UNITED STATES PATENT APPLICATION

LINKER ARMS FOR NANOCRYSTALS AND COMPOUNDS THEREOF

Be it known that we Sandra J. Rosenthal, Ian D. Tomlinson, and Tadd Kippeny, all of Nashville, Tennessee have invented new and useful linker arms for nanocrystals and compounds thereof.

Field of the Invention

This invention generally relates to nanocrystals, linker arms for nanocrystals, and compounds resulting therefrom. Furthermore, this invention relates to labeling techniques using the compounds of the present invention.

Background of the invention

Semi-conducting nanocrystals, also referred to as quantum dots, have many advantages over traditional dye molecules in the areas of fluorescent labeling. Fluorescent nanocrystal labeling has broad application in the biomedical sciences. For example, the labeling technique of the present invention provides improved and widely applicable methods for detecting biomolecules and for scrutinizing biomolecular processes.

Currently quantum dots are being used as fluorescent tags capable of tracing specific substances within cells. Quantum dots can be activated to glow with different colors, so it is easier to use quantum dots in tandem than combinations of conventional fluorescent dyes. See "Semiconductor Beacons Light up Cell Structures" Service, Science, Vol. 281. The conventional fluorescent dye, typically made from small organic dye molecules can be toxic, can quench quickly, and can be difficult to use in tandem, since typically each dye must be excited with photons at a different wavelength. Additionally, compared with conventional coloring agents such as rhodamine 6G or other organic dyes, the quantum dots produce narrower and much brighter fluorescence spectra. See "Quantum Dots Meet Biomolecules", Jacoby. With the quantum dots, or

nanocrystals, the absorbency onset and emission maxima shift to a higher energy with decreasing size. The excitation typically tracks the absorbency, resulting in a tunable fluorophore that can be excited efficiently at any wavelength shorter than the emission peak, yet will emit with the same characteristic a narrow, symmetric spectrum regardless of the excitation wavelength. See "Semiconductor Nanocrystals as Fluorescent Biological Labels", Bruchez, et al., Science, Vol. 281, 1998. The absorbance onset and emission maximum shift to higher energy as the size of the nanocrystal decreases. Because the excitation tracks absorbance, the nanocrystals can be excited at many wavelengths, yet still they emit the same narrow, symmetric peak. By varying the material used or the size of the quantum dot, the color can be changed. Additionally, a range of quantum dots of different colors may be excited with a single wavelength and detected simultaneously. See "Bright Lights for Biomolecules", Analytical Chemistry News and Features, December 1998. Thus, the quantum dots, or semiconducting nanocrystals, are much more flexible and advantageous when used in assays.

The attachment of biologically active ligands to nanocrystals including, for example, cadmium selenide nanocrystals, is a new method of producing novel fluorescent sensors. The sensors can have a variety of applications. They may be used in fundamental studies ranging from assay systems to locate the distribution and localization of membrane

bound receptors, transporter proteins and channels in whole assay systems. They may also be used in novel methodologies for the development of pharmaceutically active compounds using high throughput screening.

The small size of the of the nanocrystal ligand conjugate offers advantages over conventional techniques that use antibodies bound to fluorescent dyes. These advantages include the small size of the drug nanocrystal conjugate which enables it to fit into the synaptic gap. Antibody-fluorescent dye systems are much larger than the nanocrystal drug conjugates of the present invention, so the antibody-fluorescent dye stems are less likely to fit into the synaptic gap. Additionally most antibodies are cell permeable.

The increased photostability of the nanocrystals means that they are not as easily photo-bleached as conventional dyes. Therefore, the nanocrystal compounds of the present invention may be used in experiments that require longer periods of illumination without photo-bleaching becoming a major problem.

The increased brightness of the nanocrystals enhances the sensitivity of the assay systems when compared to traditional dyes. Therefore, assay systems can be developed that detect lower concentrations of the analyte.

Also see "Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection", Chan, Nie, Science, Vol. 281, 1998.

There are several patents that disclose nanocrystals that can be used in connection with the present invention.

U.S. Patent No. 5,990,479 to Weiss et al. discloses a luminescent nanocrystal compound that is capable of linking to an affinity molecule. Weiss et al. further describe a process for making luminescent semiconductor nanocrystal compounds and for making an organo luminescent semiconductor probe comprising the nanocrystal compound linked to an affinity molecule capable of bonding to a detectable substance and a process for using the probe to determine the presence of a detectable substance in a material.

U.S. Patent No. 5,751,018 to Alivisatos et al. discloses methods for attaching semiconductor nanocrystals to solid inorganic surfaces, using self-assembled bifunctional organic monolayers as bridge compounds.

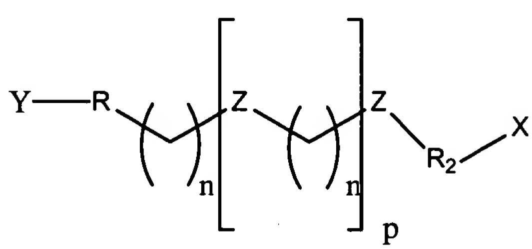
U.S. Patent No. 5,537,000 to Alivisatos et al., which describes electroluminescent devices formed using semiconductor nanocrystals as an electron transport media and a method for making such electroluminescent devices.

U.S. Patent No. 5,505,928 to Alivisatos et al. discloses nanocrystals of III-V semiconductors, and U.S. Patent No. 5,262,352

Alivisatos et al. discloses a process for forming a solid, continuos thin film of a semiconductor material on a solid support surface.

Summary of the Invention

An embodiment of the present invention is to provide linker arms to attach organic compounds to nanocrystals, or quantum dots. A linker arm of the present invention may have the following formula:



(I)

n & p = 0-10
Z = O, CH2, or NH

wherein Y represents the attachment point to the nanocrystal and X represents the attachment point of an organic compound.

R is a bond or is selected from the group consisting of:

SH,

$O(CH_{2(n)}O)_nSH$,

$NH(CH_{2(n)}O)_nSH$,

$NH(CH_{2(n)}NH)SH$,

$S(CH_{2(n)}O)_nSH$, and

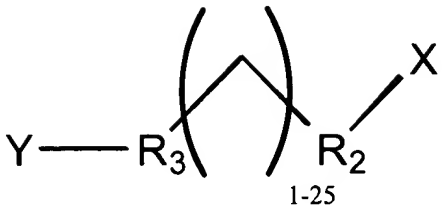
$S(CH_{2(n)}S)SH$. n is 1-10, with S being attached to the nanocrystal..

R_2 is a bond or selected from the group consisting of carbonyl, NH, S, CONH, COO, S, C_{1-10} alkyl, carbamate, and thiocarbamate.

When n and p are 1 or more, the resulting carbon or carbon chain may be substituted.

Preferably, z is O. Preferably n and p are 1-5.

In another embodiment of the present invention, the linker arm may have the following formula:



(1b)

Wherein Y is the attachment point for a nanocrystal, X is an attachment point of an organic compound.

R_2 is a bond or selected from the group consisting of

carbonyl,

O,

NH,

S,

CONH,

COO,

S,

C_{1-10} alkyl,

carbamate, and

thiocarbamate.

R_3 is selected from the group consisting of:

SH,

$O(CH_{2(n)}O)_nSH$,

$NH(CH_{2(n)}O)_nSH$,

$NH(CH_{2(n)}NH)SH$,

$S(CH_{2(n)}O)_nSH$,

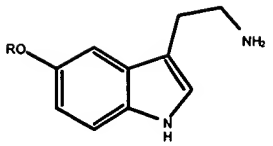
$S(CH_{2(n)}S)SH$, and

a polyether chain.

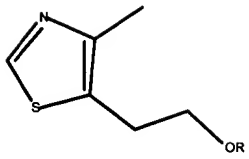
n is 1-10. S is attached to the nanocrystal.

Preferably, the organic compound is a biologically active compound. Examples of the biologically active compounds of the present invention include seratonin or seratonin derivatives, cocaine analogues, phenyl tropane analogues, phenylisopropylamine derivatives, dopamine derivatives, melatonin derivatives, chlormethiazole derivatives, derivatives of RTI-4229-75, and derivatives of GBR 12935. RTI-4229-75 and GBR 12935 are further described below.

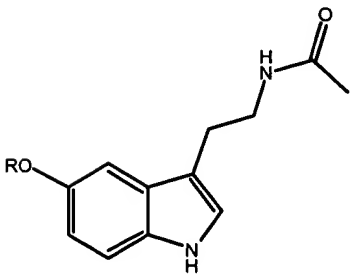
For the purposes of providing examples only, the preferred organic compounds attached to the nanocrystal of the present invention specifically include the following:



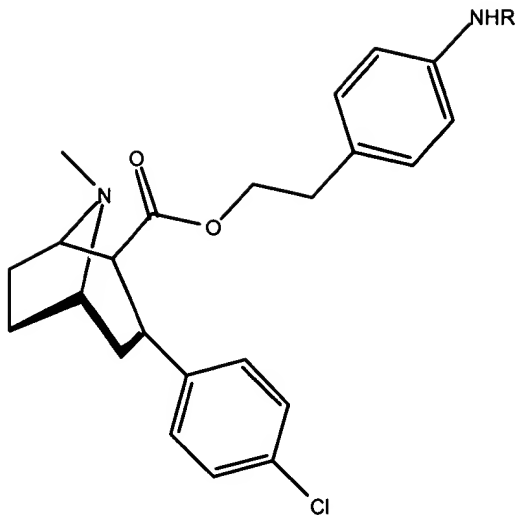
Seratonin



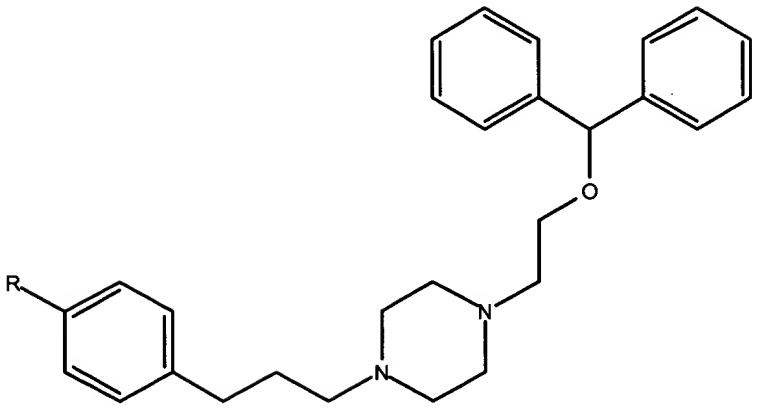
Chlormethiazole derivative



Melatonin



RTI-4229-75

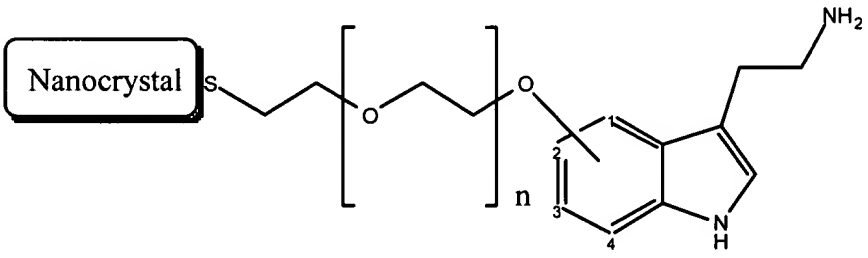


derivatives of GBR 12935

In the above examples, R represents the attachment point to the linker arm. Additionally, the R group may be "floating" when attached to the phenyl ring. That is, the R group may be attached to any available carbon atom on the ring.

The present invention further is directed to nanocrystal compounds, which include linker arm derivatives of the present invention. More specifically, the nanocrystal compounds of the present invention comprise a semiconducting nanocrystal and a linking arm having a first portion linked to the nanocrystal and a second portion linked to an organic compound.

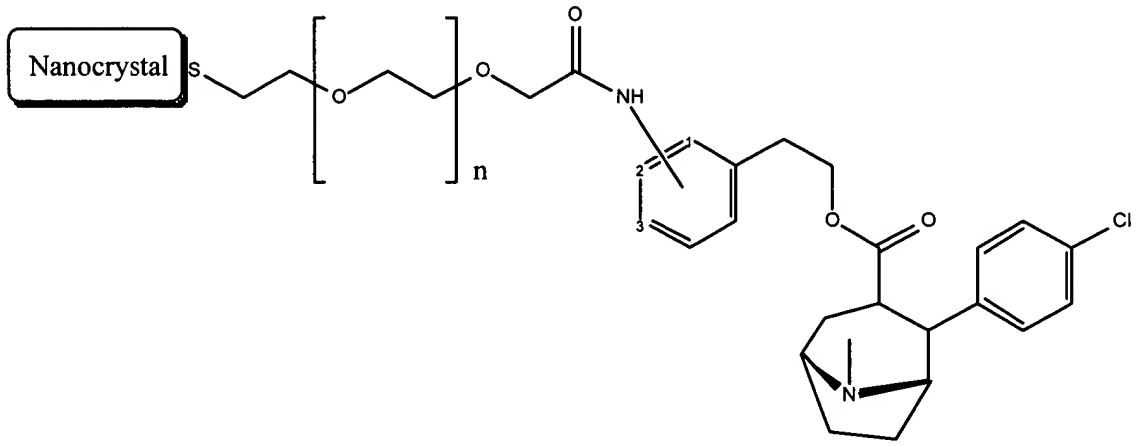
Examples of nanocrystal compounds of the present invention include the following formulae (II), (III), (IV), (V), (VI), (VII), (X) and (XI):



(II)

n = 0-10

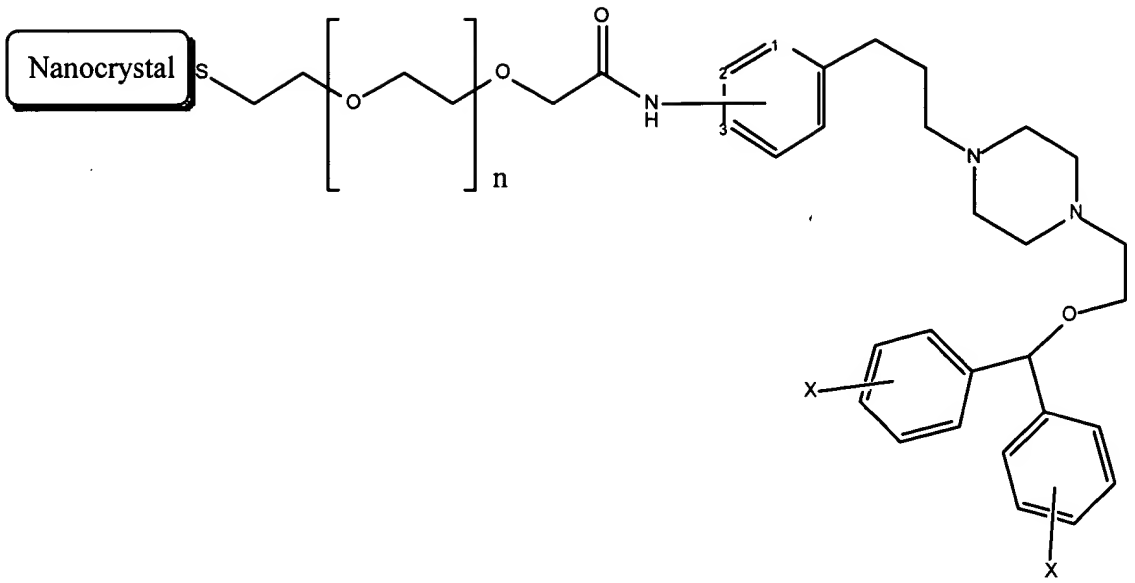
Preferably n is 2,3,4 or 5. The linker arm may be attached to positions 1,2,3, or 4. Most preferably, the linker arm is attached to position 2.



(III)

n = 0-10

Preferably n is 1, 2, 3, or 4 and the linker arm is attached to positions 1, 2, 3, or 4. Most preferably, positions 1, 2, or 3. Most preferably, position 2.

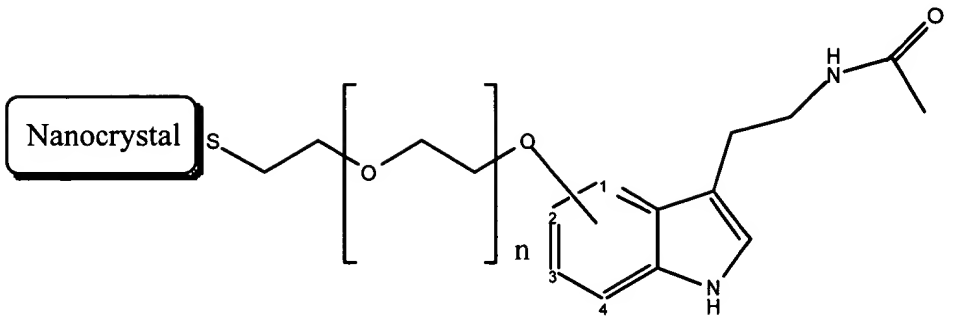


(IV)

n = 0-10

Preferably n is 1, 2, 3, 4 or 5 and the linker arm is attached to positions 1, 2, 3, or 4. Preferably, the linker arm is attached to one of positions 1, 2, or 3. Most preferably, position 3.

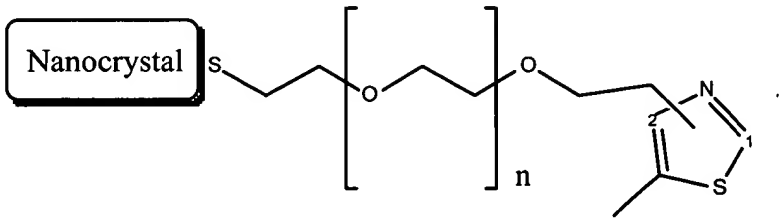
X = H or halogen. Preferably, X is H or F.



(V)

n = 0-10

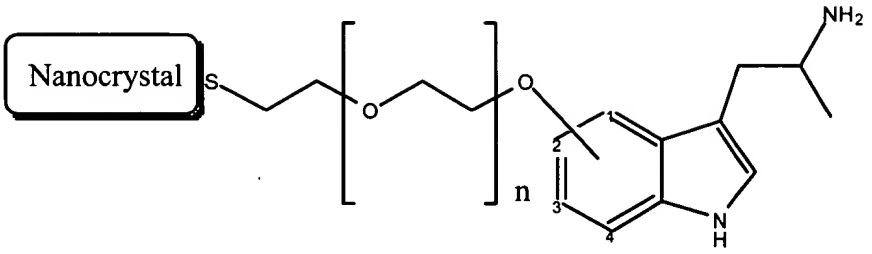
Preferably n is 2, 3, 4 or 5. The linker arm may be attached to positions 1, 2, 3, or 4. Preferably, position 2.



(VI)

n = 0-10

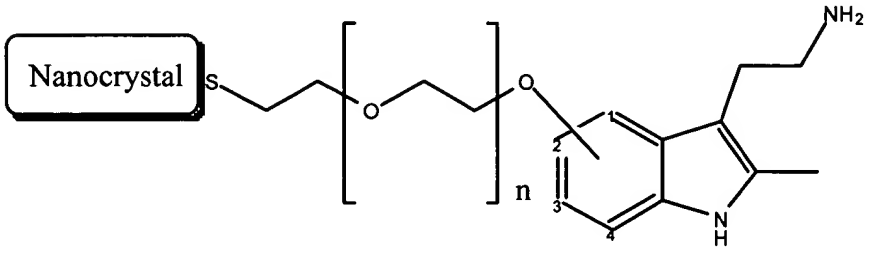
Preferably n is 2, 3, 4, or 5. The linker arm may be attached to positions 1 or 2. Preferably, position 2.



(VII)

n = 0-10

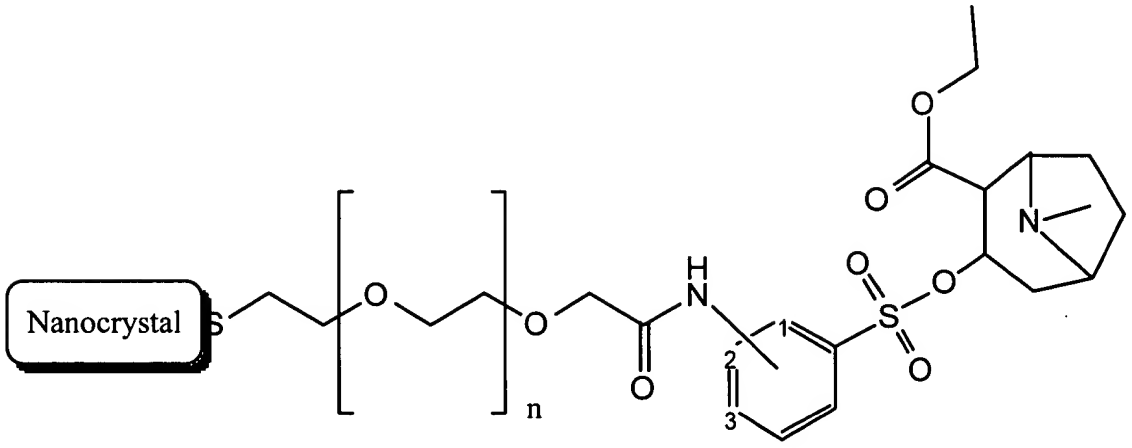
Preferably n is 2, 3, 4 or 5. The linker arm may be attached to positions 1,2,3,or 4. Preferably, position 2.



(X)

n = 0-10

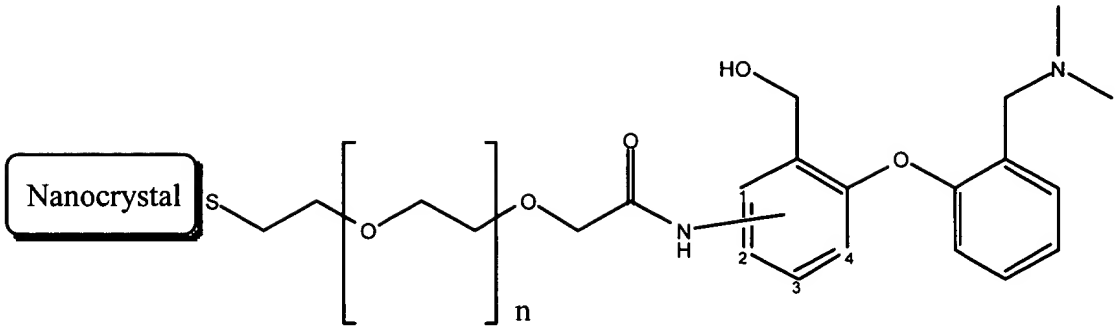
Preferably n is 2, 3, 4 or 5. The linker arm may be attached to positions 1, 2, 3, or 4. Preferably, position 2.



(XI)

n = 0-10

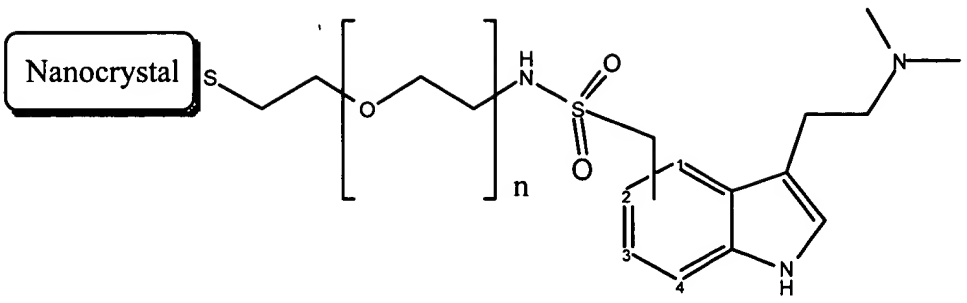
Preferably n is 2,3,4 or 5. The linker arm may be attached to positions 1,2,3,or 4. Preferably, position 2.



(XII)

n = 0-10

Preferably n is 2, 3, 4 or 5. The linker arm may be attached to positions 1, 2, 3, or 4. Preferably, position 2.



(XIII)

n = 0-10

Preferably n is 2,3,4 or 5. The linker arm may be attached to positions 1, 2, 3, or 4. Preferably, position 2.

The linker arm attaching the compounds to the nanocrystal can be altered by replacing the oxygen with sulfur or NH. The length of the alkyl substituent between the oxygen atoms may be increased or decreased and it may comprise of chains with lengths of 1 to 10 carbon atoms. Also the hetero atom in the chain may vary thus the chain may contain alternating NH and O functionalities or O and S functionalities.

Detailed Description of the Invention

As stated above, the present invention relates to linker arms to which biologically active molecules can be attached to nanocrystals. The nanocrystals used in conjunction with the present invention are the nanocrystals typically used in fluorescent imaging techniques. Preferably, the nanocrystals used in conjunction with the present invention are semiconductor nanocrystals capable of luminescence and/or scattering or diffraction when excited by an electromagnetic radiation source (of broad or narrow bandwidth) or a particle beam, and capable of exhibiting a detectable change of absorption and/or emitting radiation in a narrow wavelength band and/or scattering or diffracting when excited. For exemplary purposes, the nanocrystals of US 5,990,479 may be used with the present invention.

That is, in embodiments of the present invention, an organic or inorganic single crystal particle having an average cross-section of about 20 nanometers (nm) or 20 x 10^{-9} meters (200 Angstroms), preferably no larger than about 10nm (100 Angstroms) and a minimum average cross-section of about 1nm, although in some instances a smaller average cross-section nanocrystal, i.e., down to about 0.5nm (5 Angstroms), may be acceptable. Typically the nanocrystal will have an average cross-

section ranging in size from about 1 nm (10 Angstroms) to about 10 nm (100 Angstroms).

Furthermore, for exemplary purposes only, these nanocrystals include, but not are limited to CdSe, CdS, PbSe, PbS, and CdTe.

As mentioned above, there are disadvantages to traditional dye molecules that are used in the area of fluorescent labeling. For example, simultaneous localization of several different proteins *in situ* is currently limited by the wide emission spectra and photostabilities of fluorescent dyes traditionally used to study cell surface receptors, ion channels, and transporters. The nanocrystal compounds of the present invention can overcome the above deficiencies. For example, in one embodiment of the present invention, the nanocrystal compounds comprise core (CdSe)/shell(ZnS) semiconducting nanocrystals. Through quantum confinement, the fluorescent wavelength of these nanocrystals are continuously tunable by size. For example a 25 Angstrom nanocrystal of this embodiment emits at 455nm while a 60 Angstrom nanocrystal of this embodiment emits at 625 nm. Unlike dye molecules and variants of green fluorescent protein, these nanocrystals have narrow gaussian emission spectra enabling multiplex imaging. The absorption of these nanocrystals is continuous above the band-gap; hence all sizes of nanocrystals can be excited with a single excitation wavelength. In

addition, the nanocrystals of this embodiment are much brighter than traditional dyes, even hours after continuous illumination.

The present invention further relates to multiple organic compounds in combination with the linker arms of the present invention. The present invention further relates to a method of attaching a linker arm to multiple organic compounds and a method of attaching a linker arm to a nanocrystal. The present invention further relates to the linker arms herein described and nanocrystals attached to the linker arms herein described. The present invention also relates to nanocrystals and semiconductor nanocrystals in combination with the linker arms of the present invention. The present invention further relates to the attachment of a nanocrystal and a linker arm to an organic compound. The present invention relates to assay systems and assay kits for CNS research, receptor purification, pathogens, environmental contaminants, toxins, and screening for drugs, insecticides, herbicides, and other biologically active substances.

The linker arms and linker arm compound derivatives of the present invention enhance stability and are relatively stable, including stability to biological degradation. The linker arms and the linker arm compound derivatives of the present invention are also advantageous in that they can be synthesized at a relatively low cost.

More specifically, the present invention relates to linker arms such as, for example, ether-containing, polyether or carbon-carbon chain linker arms by which biologically active molecules such as CNS drugs and neurotransmitters can be attached to nanocrystals. The attachment of a linker arm of the present invention allows nanocrystals to be used as imaging agents in diverse applications such as biochemical research, CNS research, receptor purification, and high throughput screening for new drugs and other biologically active substances.

Additionally, the present invention relates to linker arms such as, for example, ether containing, polyether or carbon linker arm by which biologically active molecules such as drugs, hormones, etc. can be attached to nanocrystals. The linker arms of the present invention enhance water solubility of nanocrystals and allow nanocrystals to be attached to a diverse range of molecules ranging from drugs to polypeptides and neurotransmitters. The linker arm compounds of the present invention allow nanocrystals to be used as imaging agents in diverse applications such as CNS research, receptor purification, assay systems for pathogens, environmental contaminants, toxins, and a high throughput assay system for new drugs and biologically active molecules.

As stated above, preferably the organic part of the nanocrystal compounds of the present invention are biologically active compounds. Preferably, the biologically active compound is one that will bind to

detectable substances, if the substance is present, in the material being analyzed.

In general, any affinity molecule useful in the prior art in combination with a dye molecule to provide specific recognition of a detectable substance will find utility in the formation of the organo-luminescent semi conductor nanocrystal probes of the invention. Such affinity molecules include, by way of example only, such classes of substances as monoclonal and polyclonal antibodies, nucleic acids (both monomeric and oligomeric), proteins, polysaccharides, and small molecules such as sugars, peptides, drugs, and ligands. Lists of such affinity molecules are available in the published literature such as, by way of example, the "Handbook of Fluorescent Probes and Research Chemicals", (sixth edition) by R.P Haugland, available from Molecular Probes, Inc.

As stated above, the compounds of the present invention enable nanocrystals to be used as probes for neurotransmitters, receptors and transporter proteins. In one embodiment of the present invention, seratonin (5-hydroxytriptamine) is attached to a nanocrystal. Seratonin is a neurotransmitter which has been linked to the regulation of critical behaviors including sleep, appetite, and mood.

The seratonin transporter (SERT) is a 12-transmembrane domain protein responsible for the uptake of seratonin by the cell. The seratonin

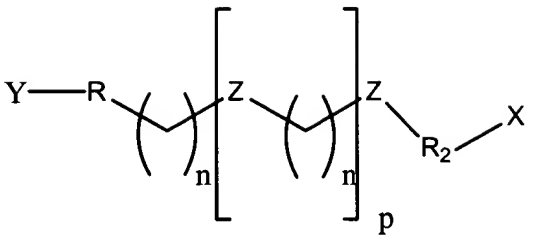
labeled nanocrystal compounds of the present invention have a measurable ability to block the uptake of tritiated sepatonin by the human and *Drosophila* seratonin transporter (hSERT and dSERT).

Seratonin labeled nanocrystals (SNACs) of the present invention may be prepared by reacting trioctylphosphineoxide coated nanocrystals with seratonin and tetramethylammonium hydroxide in methanol. The SNACs are isolated by precipitation and purified to remove seratonin. Linkage of the seratonin presumptively occurs through the lone pair of the hydroxyl to the Cd surface atoms of the nanocrystal. hSERT and dSERT are transfected into HeLa cells via a vaccinia virus/T7 expression system. Following expression of the transfected transporters, the cells are assayed for uptake of tritiated seratonin in the presence of increasing concentrations of SNACs. K_i values, the concentration at which half the SNACs are bound to the transporter, are determined by nonlinear regression. The values [K_i (hSERT) = 74uM, Ki (dSERT) = 29uM] indicate SNACs can effectively interact with the seratonin recognition site of the transporter.

These results suggest that highly fluorescent, seratonin labeled nanocrystals can be used as probes for SERT. These probes assist in determining the structure of SERT, including the number of gene products (SERT proteins) that are required to assemble a functional unit, and following transporter movement within the cell.

The present invention enables nanocrystals to be used as imaging agents, which results in an assay system that is superior to traditional immunoassay systems because, among other things, several wavelengths can be used to induce fluorescence. The linker arm can be attached to a number of different ligands, thus enabling them to be used in high throughput screening and receptor purification. The linker arm is stable and not as subject to enzymatic degradation as other linker arms may experience. The linker arm of the present invention also enhances the solubility of the nanocrystal, and can be readily derivitised. This enables a wide range of molecules to be attached to the nanocrystals. The linker arm of the present invention is not as temperature sensitive as many immunoassay systems, and thus is likely to have a longer shelf life. Further, the linker arm of the present invention is also robust and therefore not susceptible to extremes of pH that may denature and degrade peptide linkers.

As stated above, the linker arm of the present invention may have the following formula:



(I)

n & p = 0-10
Z = O, CH2, or NH

wherein Y represents the attachment point to the nanocrystal and X represents the attachment point of an organic compound.

R is a bond or is selected from the group consisting of:

SH,

$O(CH_{2(n)}O)_nSH$,

$NH(CH_{2(n)}O)_nSH$,

$NH(CH_{2(n)}NH)SH$,

$S(CH_{2(n)}O)_nSH$, and

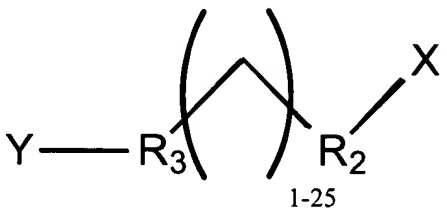
$S(CH_{2(n)}S)SH$. n is 1-10, with S being attached to the nanocrystal..

R_2 is a bond or selected from the group consisting of carbonyl, NH, S, CONH, COO, S, C_{1-10} alkyl, carbamate, and thiocarbamate.

When n and p are 1 or more, the resulting carbon or carbon chain may be substituted.

Preferably, z is O. Preferably n and p are 1-5.

In another embodiment of the present invention, the linker arm may have the following formula:



(1b)

Wherein Y is the attachment point for a nanocrystal, X is an attachment point of an organic compound.

R_2 is a bond or selected from the group consisting of

carbonyl,

O,

NH,

S,

CONH,

COO,

S,

C_{1-10} alkyl,

carbamate, and

thiocarbamate.

R_3 is selected from the group consisting of:

SH,

$O(CH_{2(n)}O)_nSH$,

$NH(CH_{2(n)}O)_nSH$,

$NH(CH_{2(n)}NH)SH$,

$S(CH_{2(n)}O)_nSH$,

$S(CH_{2(n)}S)SH$, and

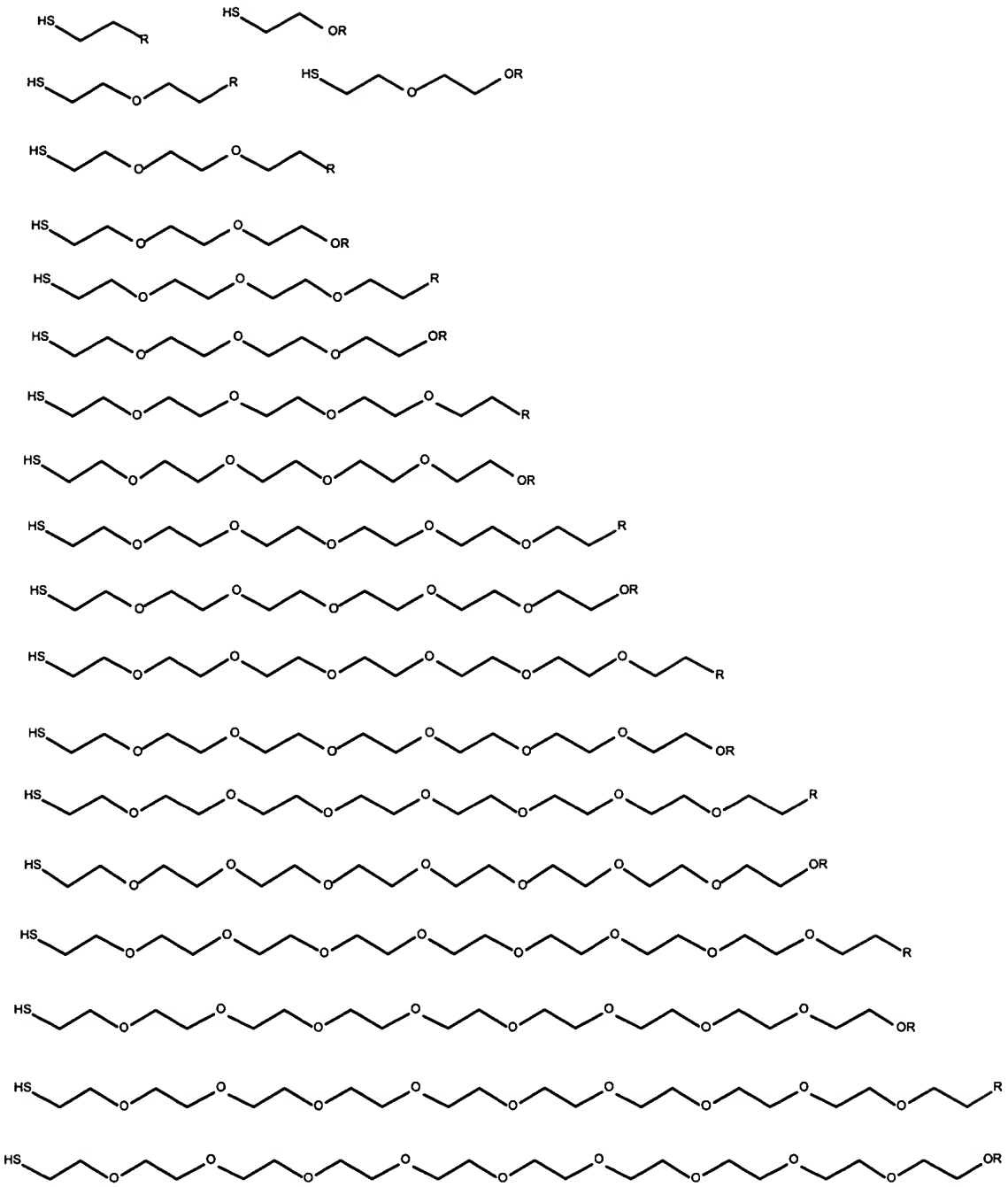
a polyether chain.

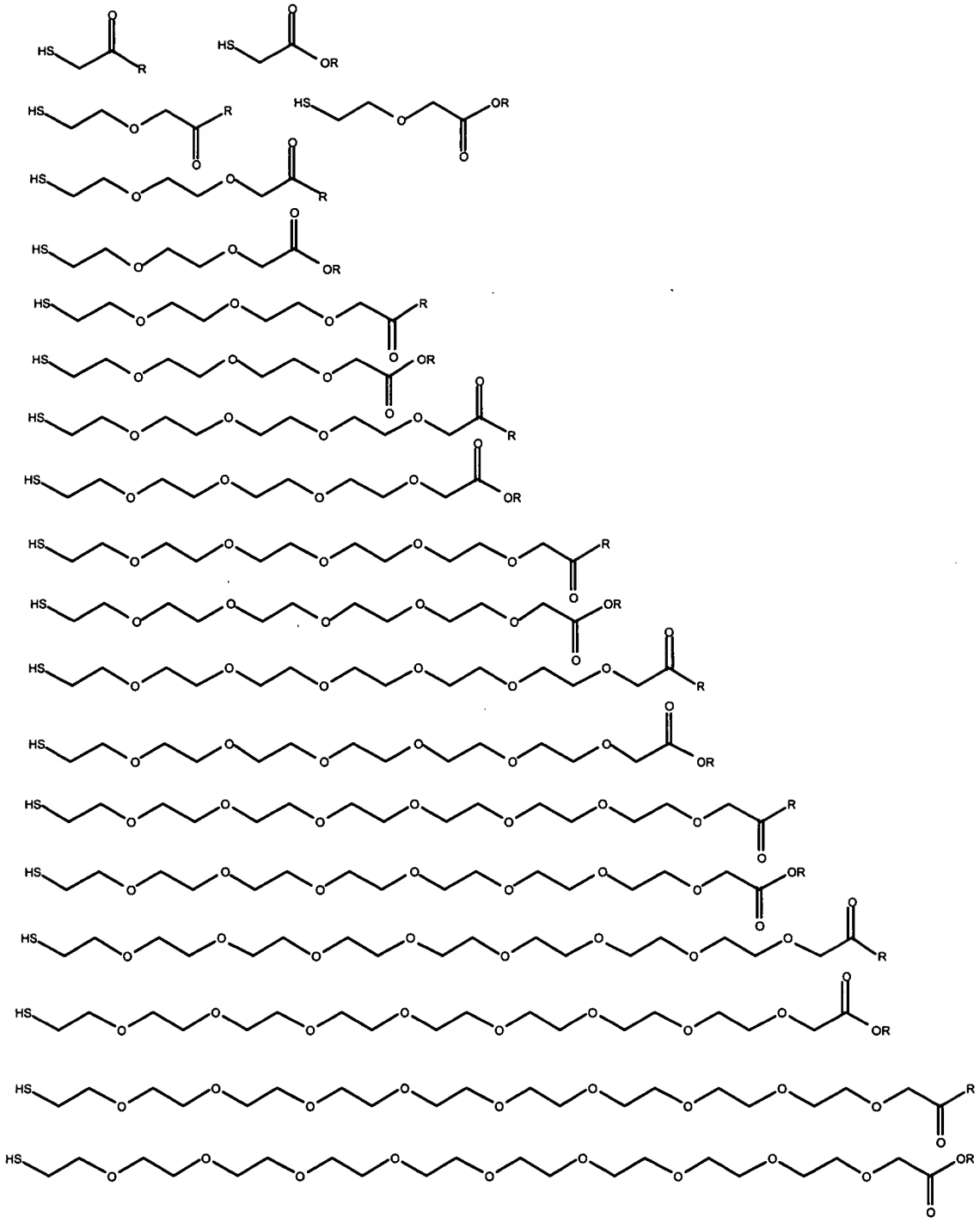
n is 1-10. S is attached to the nanocrystal.

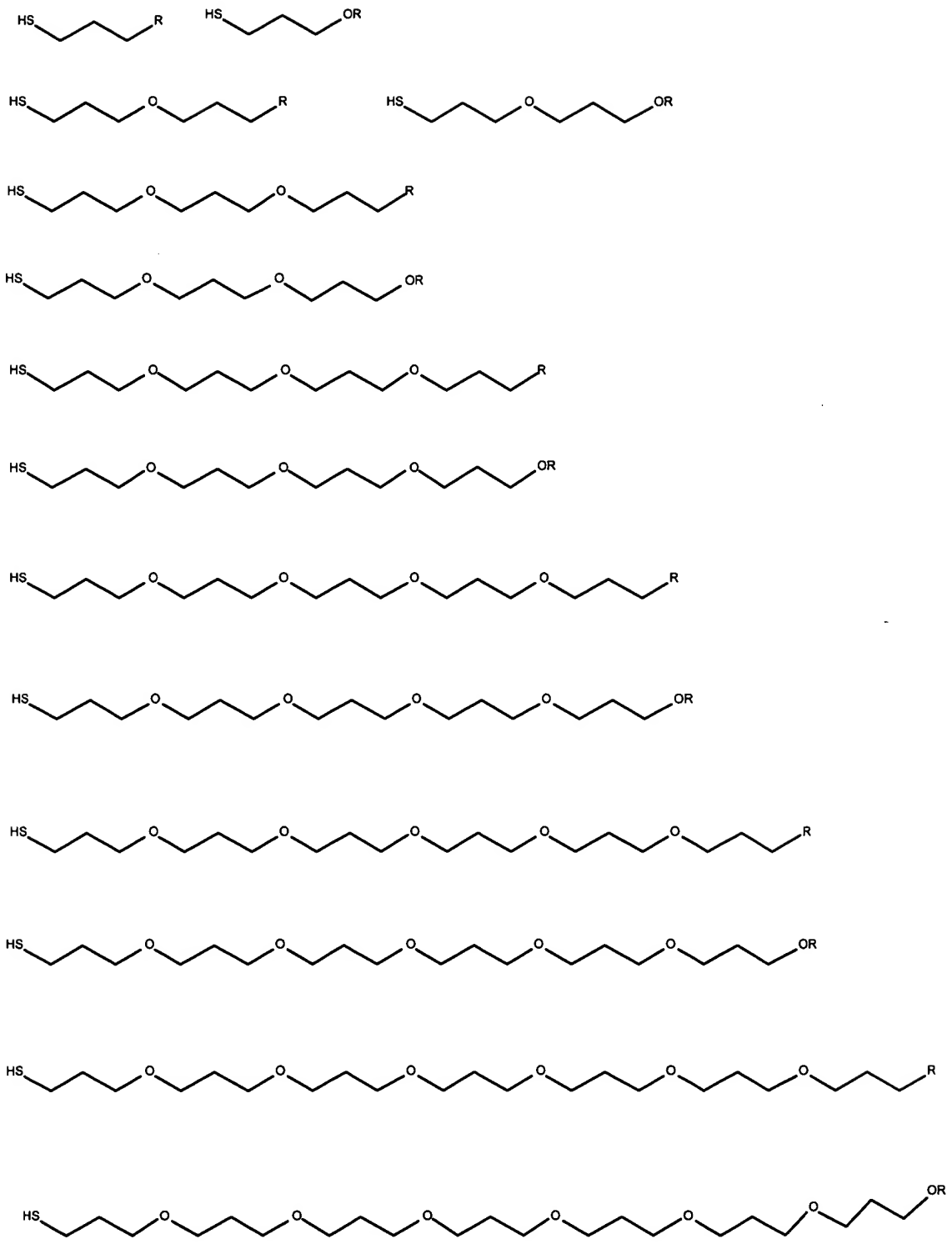
Preferably, n is 1-5.

The length of the linker arms of the present invention may be increased or shortened in order to increase the solubility of the nanocrystal drug conjugate and increase the affinity of the ligand for its target protein.

The linker arms of the present invention include the following compounds:






HS
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R
HS
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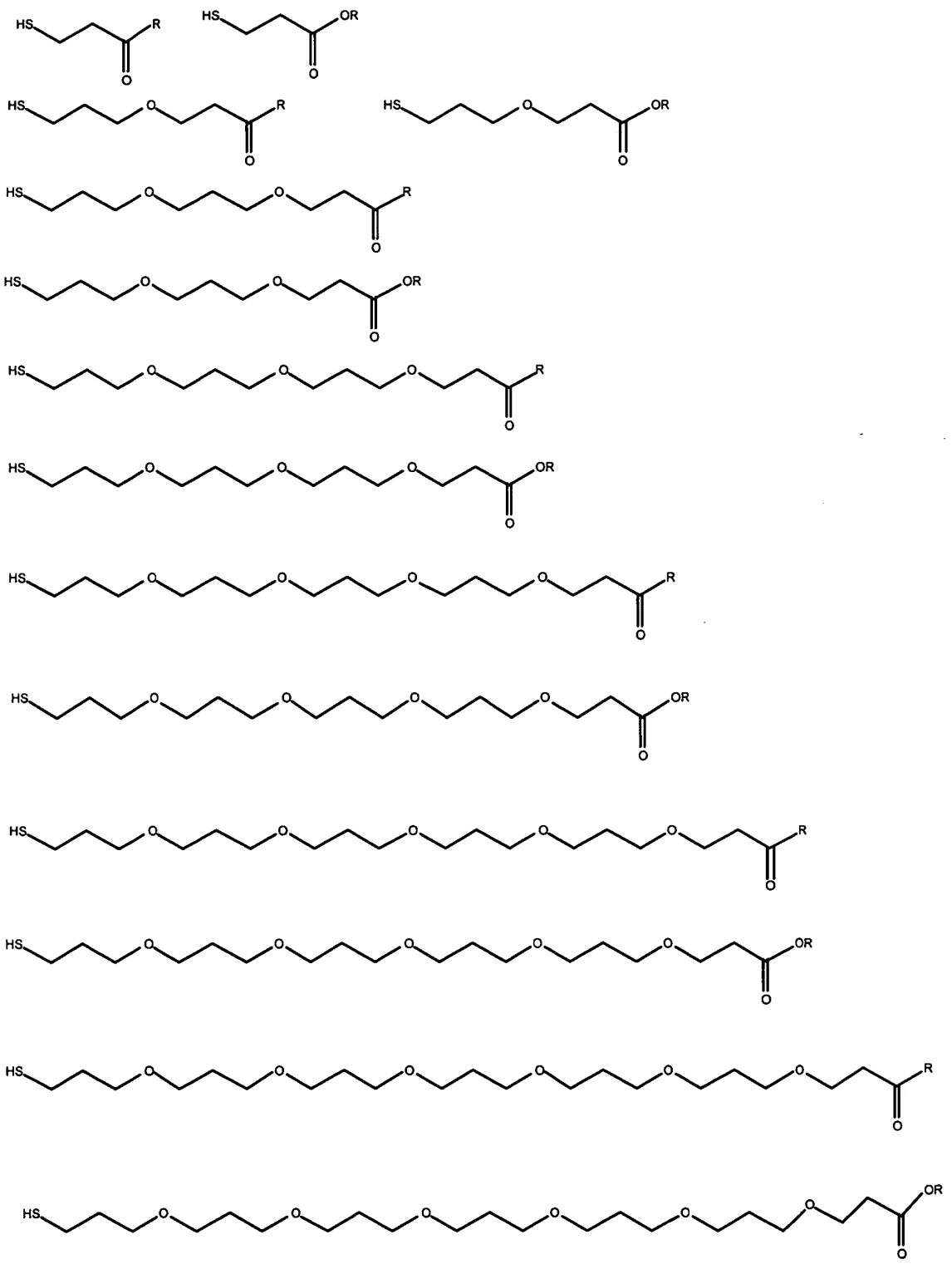
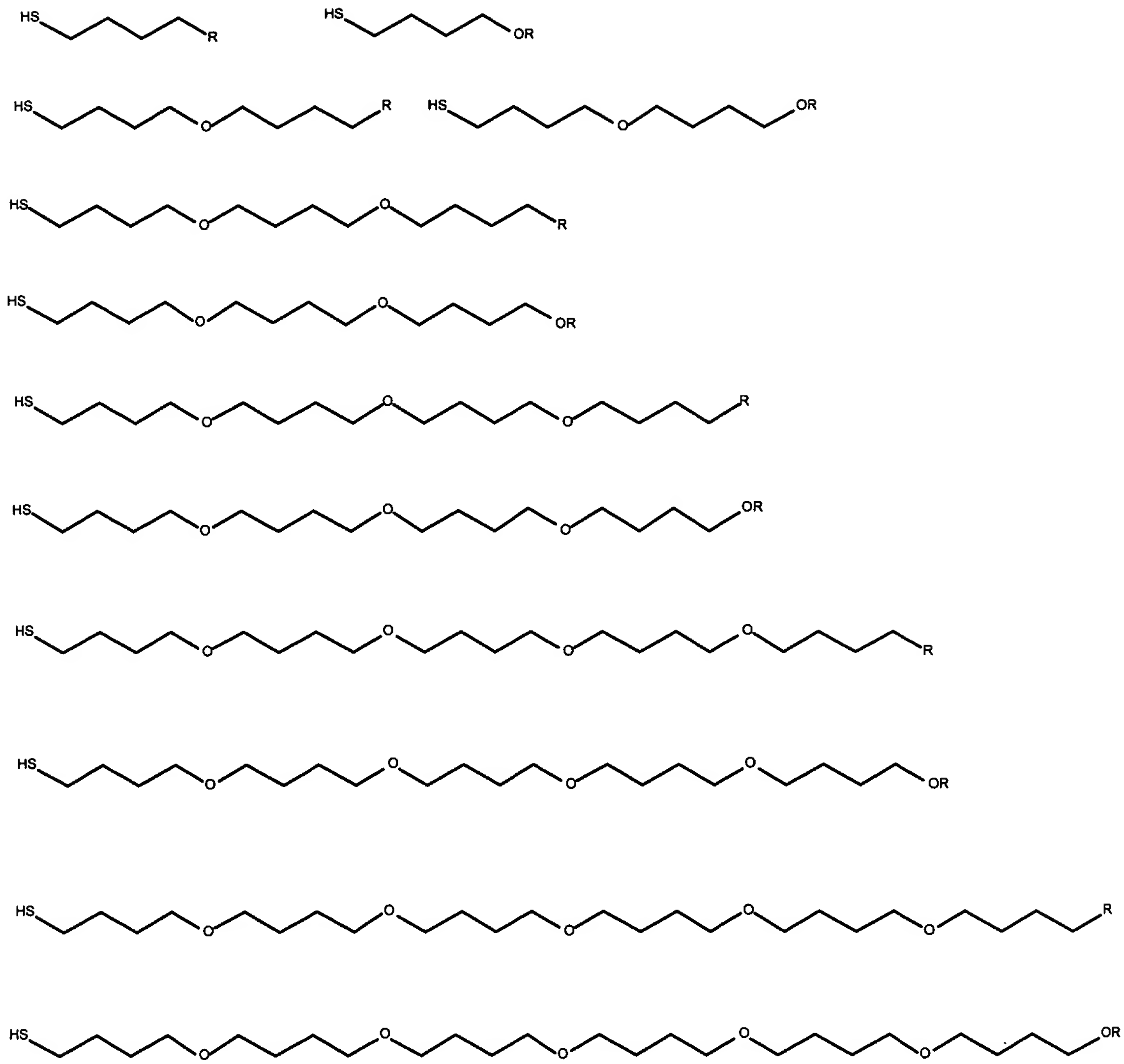
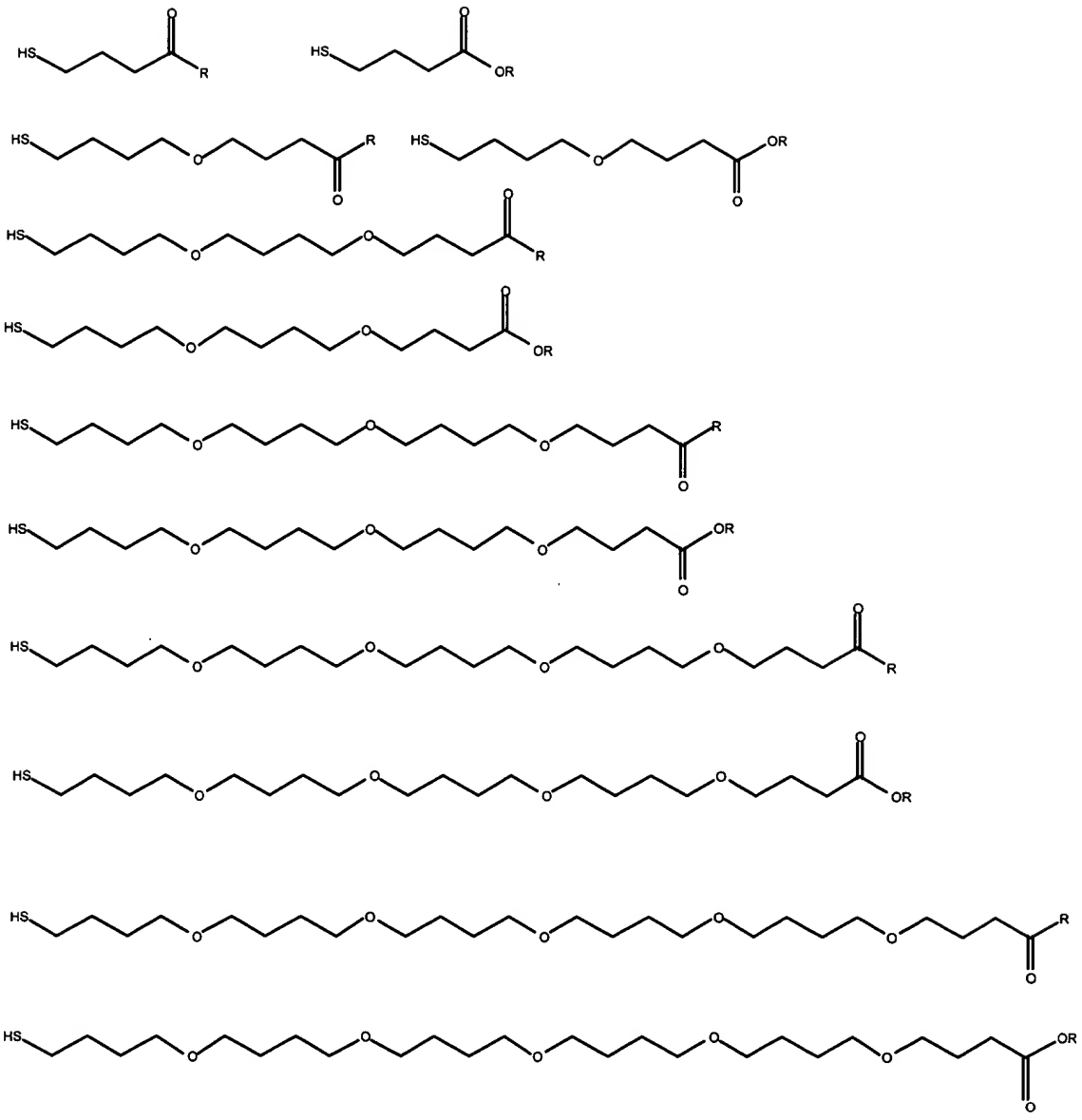
OR







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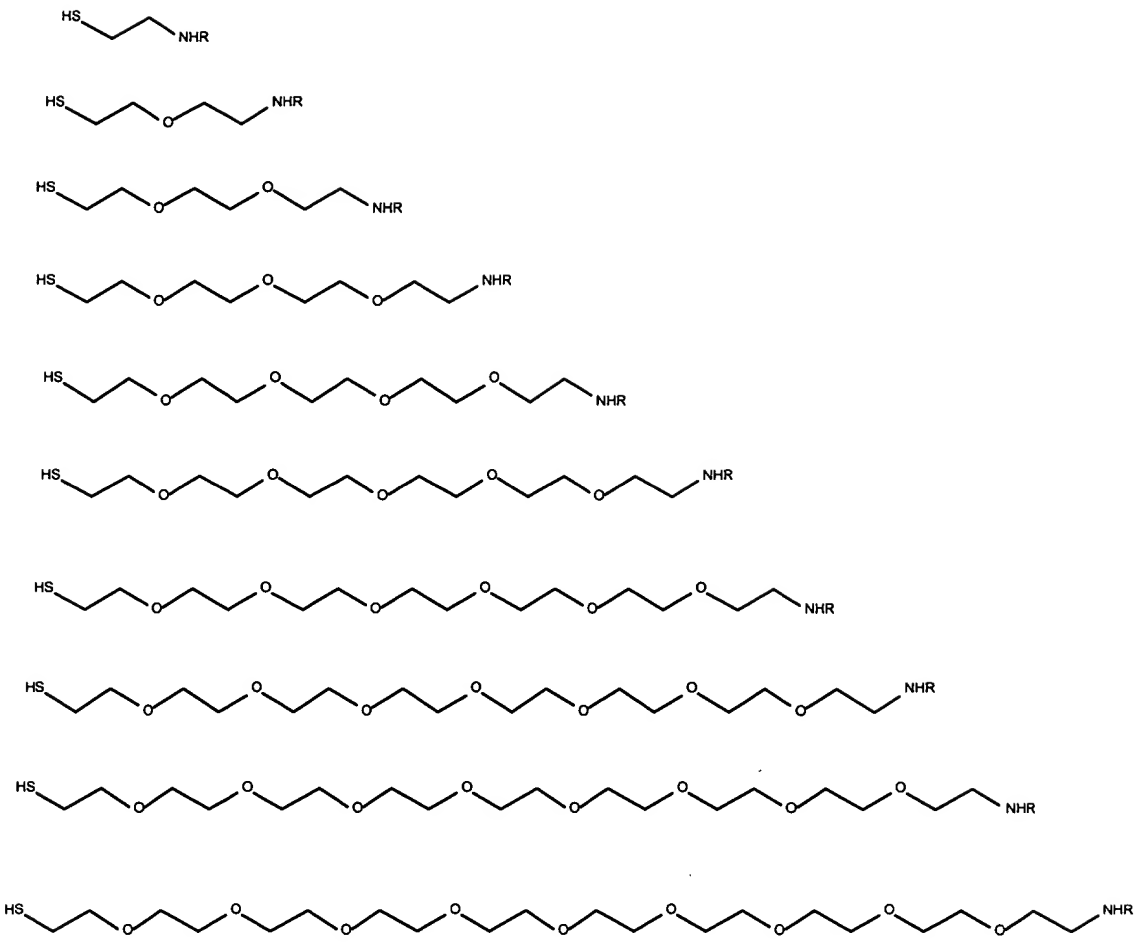
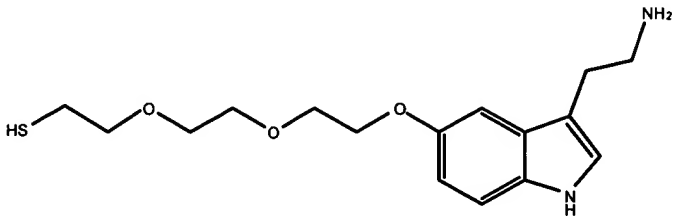
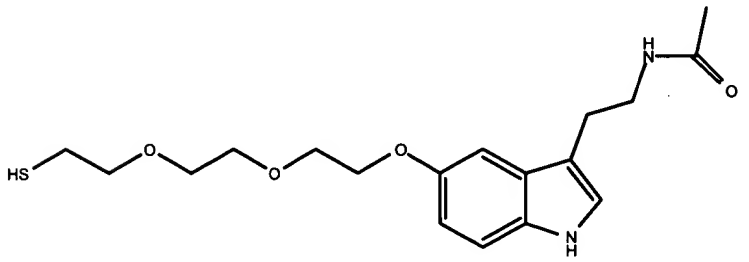
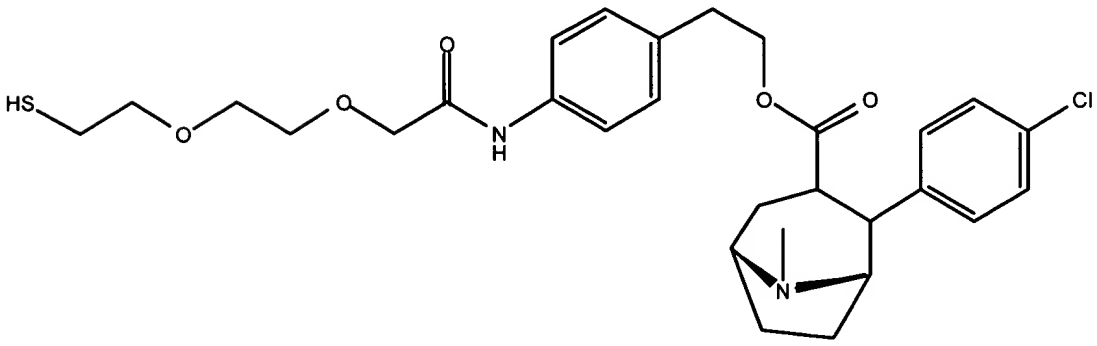


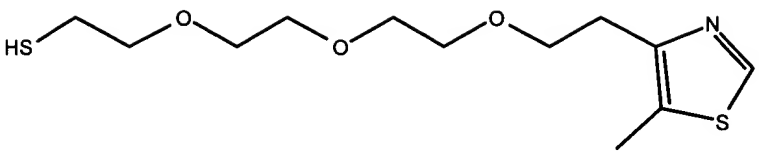
In the above examples, R represents the point of attachment of an organic compound.

The nanocrystal compounds of the present invention include the following examples, with S being the attachment point of the nanocrystal:









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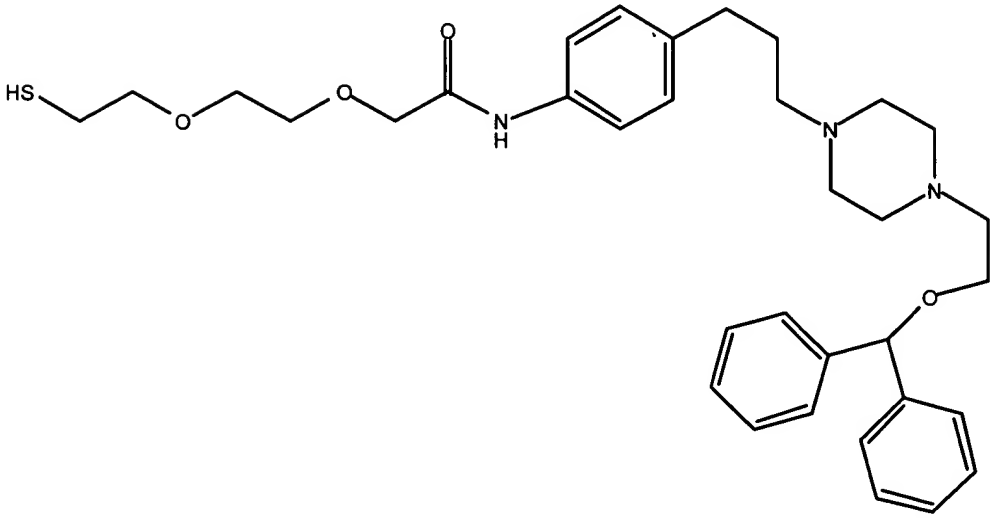

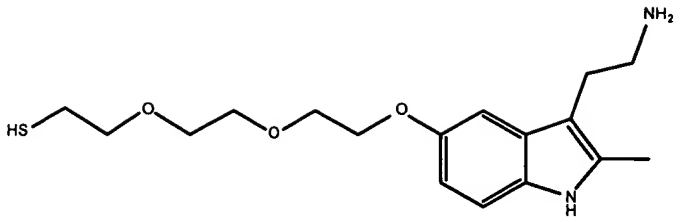
NH2
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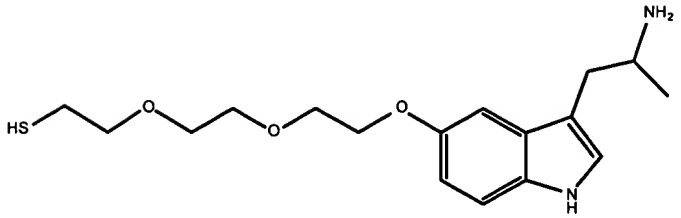
N
H


NH2
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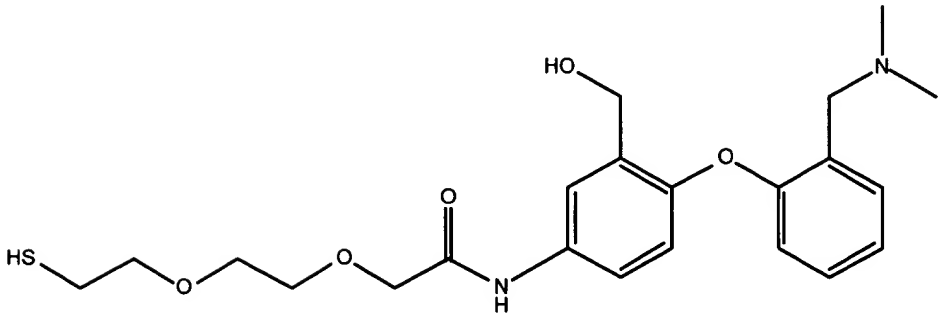
N
H


HO
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HS
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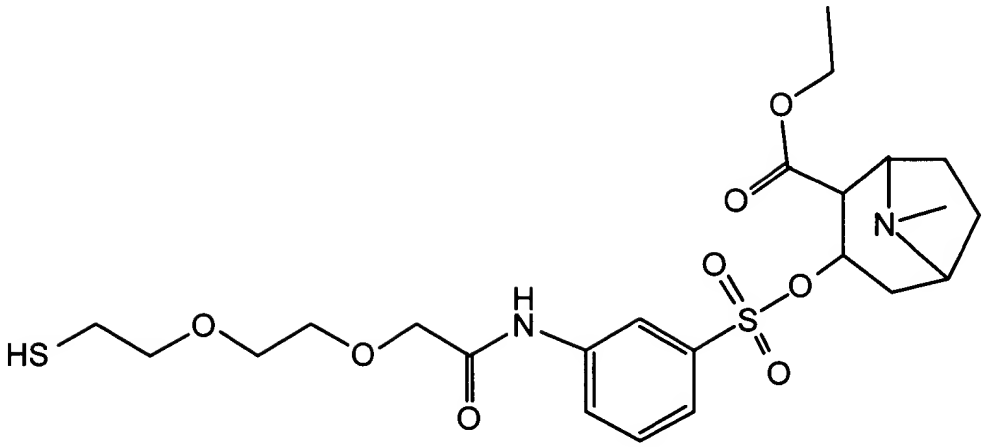
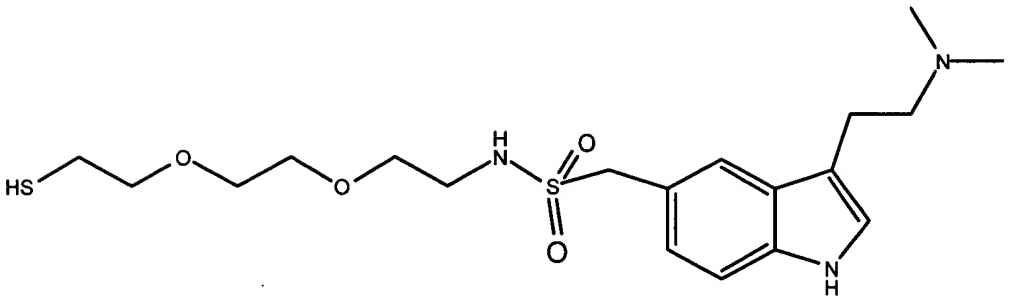




Nanocrystal compounds of the present invention include compounds that comprise of nanocrystals with the following specific and preferred features: a CdSe core, ZnS shell, generally their cores are less than 25nm, in diameter. The surrounding ZnS shell is typically 10 to

20nm in thickness, and the ligand coated core shells are water solubilized by the addition of a mercapto acetic acid co-solubility ligand.

By attaching antibodies to nanocrystals via a linker arm of the present invention, nanocrystals can be made to bind to specific antigens. Accordingly, an embodiment of the present invention is an assay kit developed for the detection of a diverse range of substances ranging from environmental contaminant such as DDT, dioxanes, chemical warfare agents, herbicides, pesticides, and pathogenic organisms such as Ecoli 0157 and Salmonela.

For example, the present invention comprises a process for treating a material, such as a biological material, to determine the presence of a detectable substance in the material. The process comprises contacting the material with a nanocrystal conjugated compound of the present invention, washing unbound nanocrystal conjugated compound away, and exposing the material to energy such as an electromagnetic source or particle beam capable of exciting the nanocrystal conjugated compound of the present invention, and causing a detectable fluorescence to occur in the nanocrystal conjugated compound of the present invention. Thus enabling the location and distribution of a particular substance within the biological material to be determined.

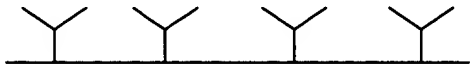
The nanocrystal compounds of the present invention may be used in the assays described in US Patent 5,990,479.

One assay system of the present invention is a high throughput fluorescence assay to identify novel ligands that might be effective antidepressants or ligands that might help combat cocaine addiction. In this assay a known agonist or antagonist for the dopamine receptor or transporter is bound to nanocrystals, and incubated with cells that either naturally express or have been engineered to express dopamine receptors or transporters. After incubating for 12 hours excess ligands are removed by washing and unknown compounds are incubated with the cells for a further 12 hours. The cells are washed again with buffer and a fluorescence assay is performed. Any cells that no longer fluorescence have a high affinity ligand bound to them and this ligand may be used as a lead compound for drug discovery. Such an assay system may be carried out in a conventional multiple well format system, such as the 96 well format.

Chart A, below demonstrates another method of the present invention that may be used to detect biologically active analytes. Chart A describes a sandwich assay system. In chart A, in step 1 monoclonal or polyclonal antibodies raised against a specific analyte or groups of analytes are bound to the surface of the plate. In step 2, the analyte is added and binds to the antibody. In step 3, the unbound analyte is washed away and a nanocrystal antibody conjugated using our linker arm of the present invention is added (once again poly or monoclonal

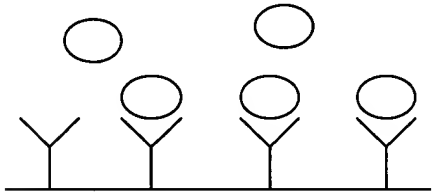
antibodies may be used). In step 3, the unbound nanocrystal antibody conjugates are removed by washing, and a fluorescence assay is performed to determine if the analyte is present in the sample being analyzed and its concentration as a sample with a higher concentration will produce a greater fluorescence. Multiple analytes can be screened for using a conventional 96 well plate format.

(1)



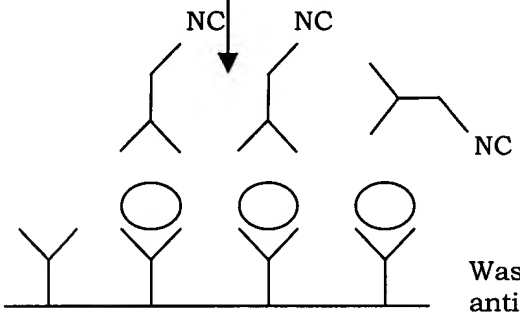
Monoclonal or polyclonal antibodies raised against a specific analyte or group of analytes are bound to a surface.

Analyte is added



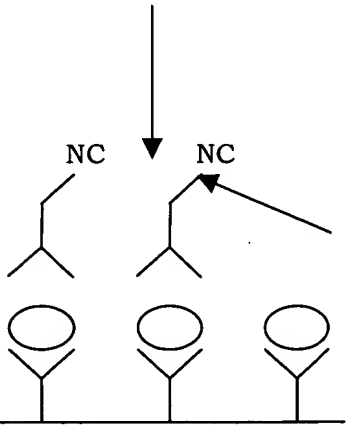
Analyte binds to the antibody.

Wash to remove unbound analyte and add our nanocrystal antibody conjugate



(3)

Wash to remove unbound nanocrystal antibody conjugate and perform fluorescence assay.



Polyethylene glycol linker arm

(4)

Chart A. NC = nanocrystal. A sandwich assay system.

The nanocrystal of the present invention may be used in affinity chromatography, where a compound or biological molecule of interest may be bound to a column. This may then be specifically labeled with the antibody nanocrystal conjugate, substrate nanocrystal conjugate, or drug nanocrystal conjugate of the present invention. The compound could be a drug, a hormone, an enzyme, a protein, a nucleic acid or a receptor. Once the nanocrystal conjugate has bound to the substrate of interest, it may either remain bound to the column or be eluted with the mobile phase. This would enable the isolation and identification of the compound orbiological molecule of interest. Unlike fluorescent dyes, nanocrystals are not easily photo-bleached. Therefore, it would be easier to watch the compound or compounds eluting off the column. Also such a system may be applied to several different analytes enabling the identification of several unknowns at once by using different sized nanocrystals conjugated to different ligands. Thus it is theoretically possible to identify different receptor classes or subtypes (e.g. 5-HT receptor subtypes) as they elute off the column. For example it may be possible to differentiate between 5HT2 and 5HT3 receptor subtypes using such a system.

The linker arm acts as a spacer and separates the ligand from the nanocrystal thus possible steric and other interactions between nanocrystals and ligand are minimized. The linker arm may be an ethylene glycol moiety this helps to enhance the solubility in aqueous media. Many affinity chromatographic systems are typically run in such media. The polyether linker arm is also resistant to proteolytic cleavage which may be a problem with other assay systems.

Nanocrystals can be attached to enzymes via linker arms of the present invention. Thus the amino derived carboxylic acid derived poly-ethers may be linked to the backbone of the peptide via a peptide bond.

In this instance the linker arm removes the enzyme from the immediate environment of the nanocrystal. This may be important in reducing any effects that the nanocrystal may have upon the enzymes activity. Many such instances could be envisaged particularly if the enzyme or protein undergoes a conformational change during its catalytic cycle (e.g. Hemoglobin). Also the linker arm may increase the catalytic efficiency of the enzyme if the active site or sites are close to the enzymes surface.

Such a system may also be used to identify analytes in a similar manner to the nanocrystal antibody conjugates previously described. It may also be used in high throughput screening where the compounds of

interest are bound to wells in plates and the enzyme nanocrystal conjugate is added. An example of this is shown in chart B below:

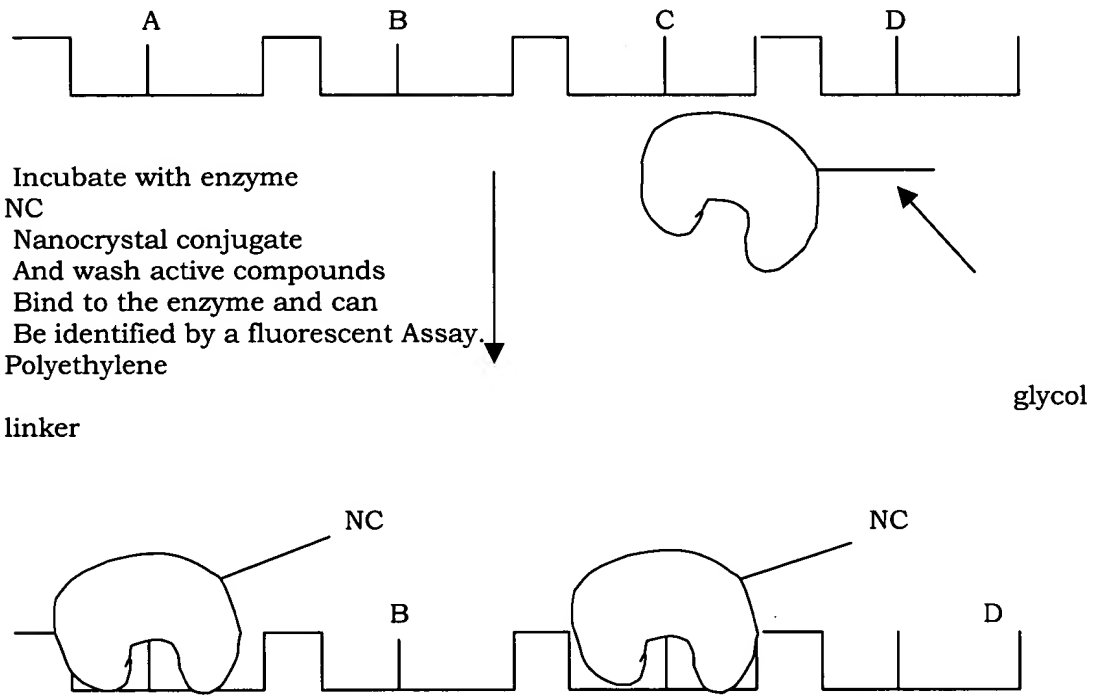


Chart B identifying active compounds in a high throughput assay system

The enzymes substrate or inhibitor may also be bound to the polyethylene glycol nanocrystal conjugate. In this instance, the linker arm of the present invention reduces steric hindrance between

nanocrystal and enzyme and it enables the substrate to enter the enzymes catalytic or alosteric site, which may not be possible if the substrate were bound to the surface of the nanocrystal (particularly if the site of interest is deep within the enzyme). An assay system that could use this technique as a tool for identifying new drugs is outlined in chart C, below, where compounds that will compete for the site of interest can be identified. If the nanocrystal is bound to an inhibitor via the linker arm of the present invention it is likely that this assay system could also be used to identify other inhibitors of the enzyme.

Chart C:

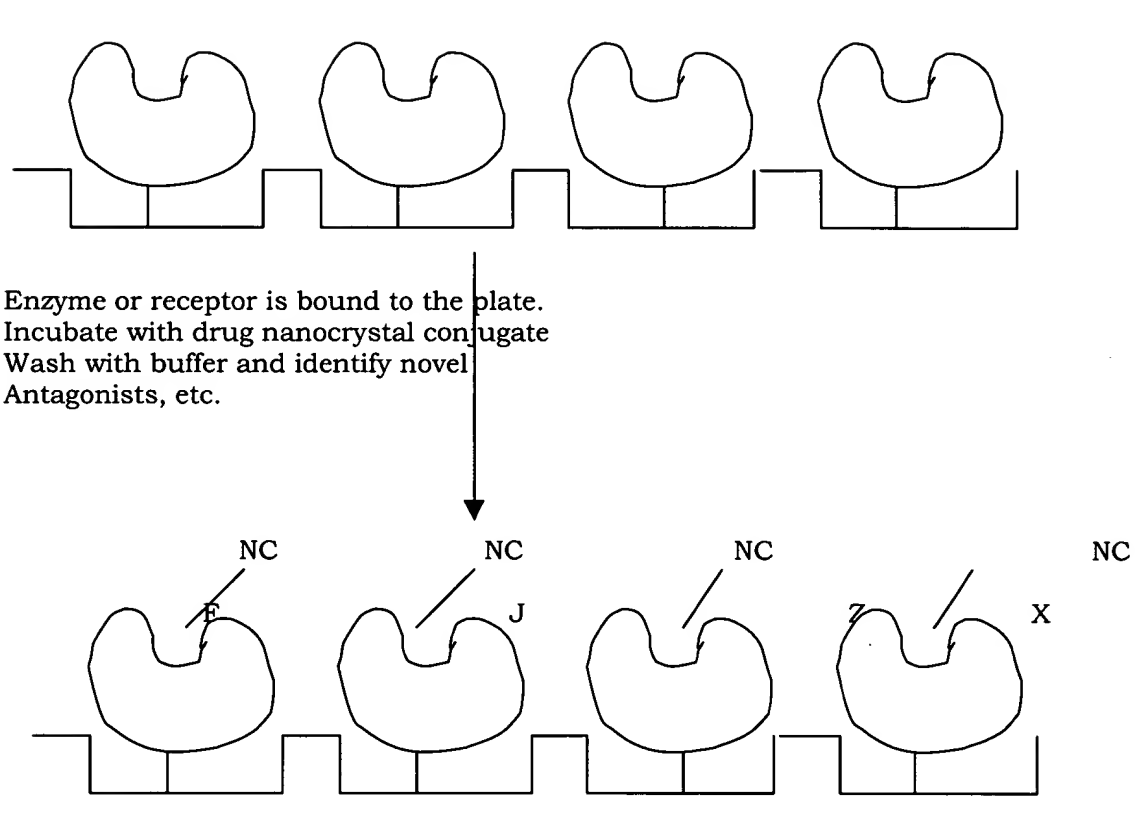


Chart C.

F,J,X and Z are new chemical entities bound to nanocrystals via our linker arm. Inactive compounds will not bind and will be washed away thus we have an assay system for detecting novel active compounds.

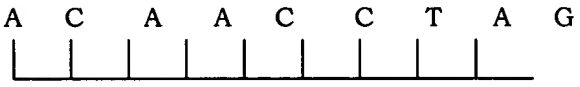
One specific substance may also be bound to the nanocrystal (e.g. a substrate for the enzyme) and a simple competitive assay could be performed with unknown substances in a manner similar to that shown

above in chart C. Any substance that has a higher affinity for the site of interest on the enzyme, protein or receptor than the ligand conjugated nanocrystal would displace the ligand conjugated nanocrystal resulting in a loss off fluorescence, thus enabling this system also to be used as a high throughput assay system as well as an analytical tool for environmental contaminates, toxins, and other unknowns.

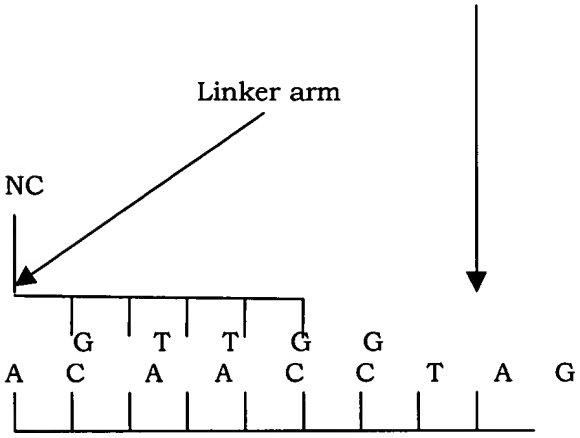
This system can be applied to receptors rather than enzymes. In this case, the nanocrystal is bound to an agonist, antagonist, or natural ligand for the receptor (e.g. Seratonin). This system could be used as an assay system for receptor agonist or antagonist. It would be of interest in neuropharmacology where receptor location and distribution could be mapped. By attaching different sized nanocrystals to different agonists, antagonists, or ligands it may be feasible to develop multiplexing assay systems, thus enabling the effects of drugs and other neurologically active agents to be monitored in whole cell assay systems. Assaying the location and distribution of many membrane bound receptors and transporter proteins is currently difficult using conventional antibody fluorescent dye systems is difficult due to photo-bleaching and the broader emission spectra of dyes.

Nanocrystals may be attached to DNA or RNA via the linker arm of the present invention. In this case, the major role of the linker arm acts as a spacer and reduces steric hindrance. The DNA or RNA conjugates

may be used as a tool in molecular biology for identifying the location and frequency and rate of expression of specific gene sequences. Such a system is outlined in chart D, below:



Incubate DNA or RNA with our strand of labeled RNA or DNA that is conjugated to A nanocrystal via the linker arm. Then wash. The sections of the DNA or RNA of interest will bind to our probe.



Thus the distribution and location of genes can be shown also the frequency of the gene in DNA or RNA can be measured.

Chart D.

The nanocrystal conjugates of the present invention can also be used in assay systems in the same manner that antibody fluorescent dye conjugates, radio immuno assays, and ELISA are used. Examples of the assay system include routine assays used in medical laboratories such as tests for various disease states for example HIV, Diabetes, etc.

Other features of the invention will become apparent in the course of the following examples which are given for illustration of the invention and are not intended to be limiting thereof.

Examples

Ligands That Comprise of a Biologically Active Molecule

Two synthetic routes have been developed for the production of a seratonin derivative with a polyether linker arm attached to it. These routes are outlined in charts 1, 2, and 3. Route B is a variant of Route A in which the protecting group is changed to a phalimido substituent.

Preparation of the linker arm

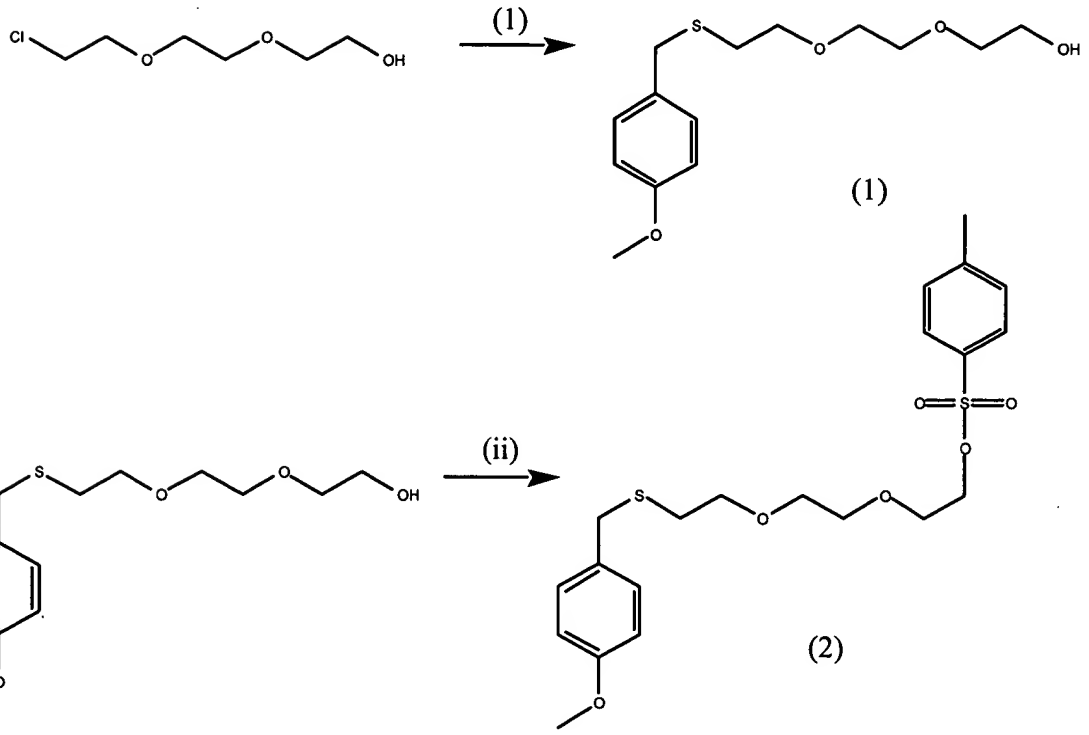
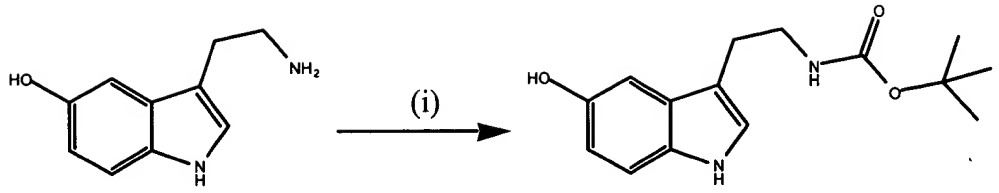


Chart 1:

(i) 2-(2-(2-Chloroethoxy)ethoxy)ethanol, Sodium, ethanol, paramethoxy-α-toluene thiol, reflux 24 hours; (ii) 1, pyridine, para-toluene sulfonyl chloride, stir at room temperature for 24 hours.



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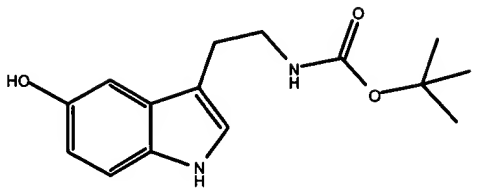
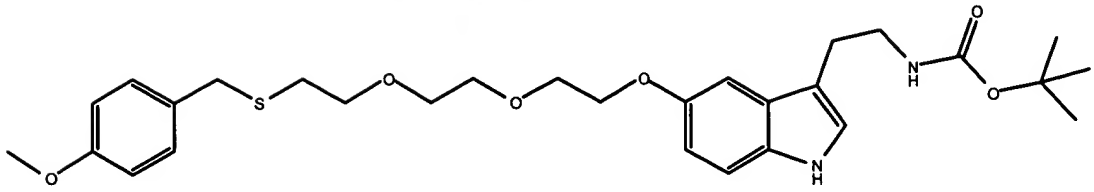


(3)

(ii)



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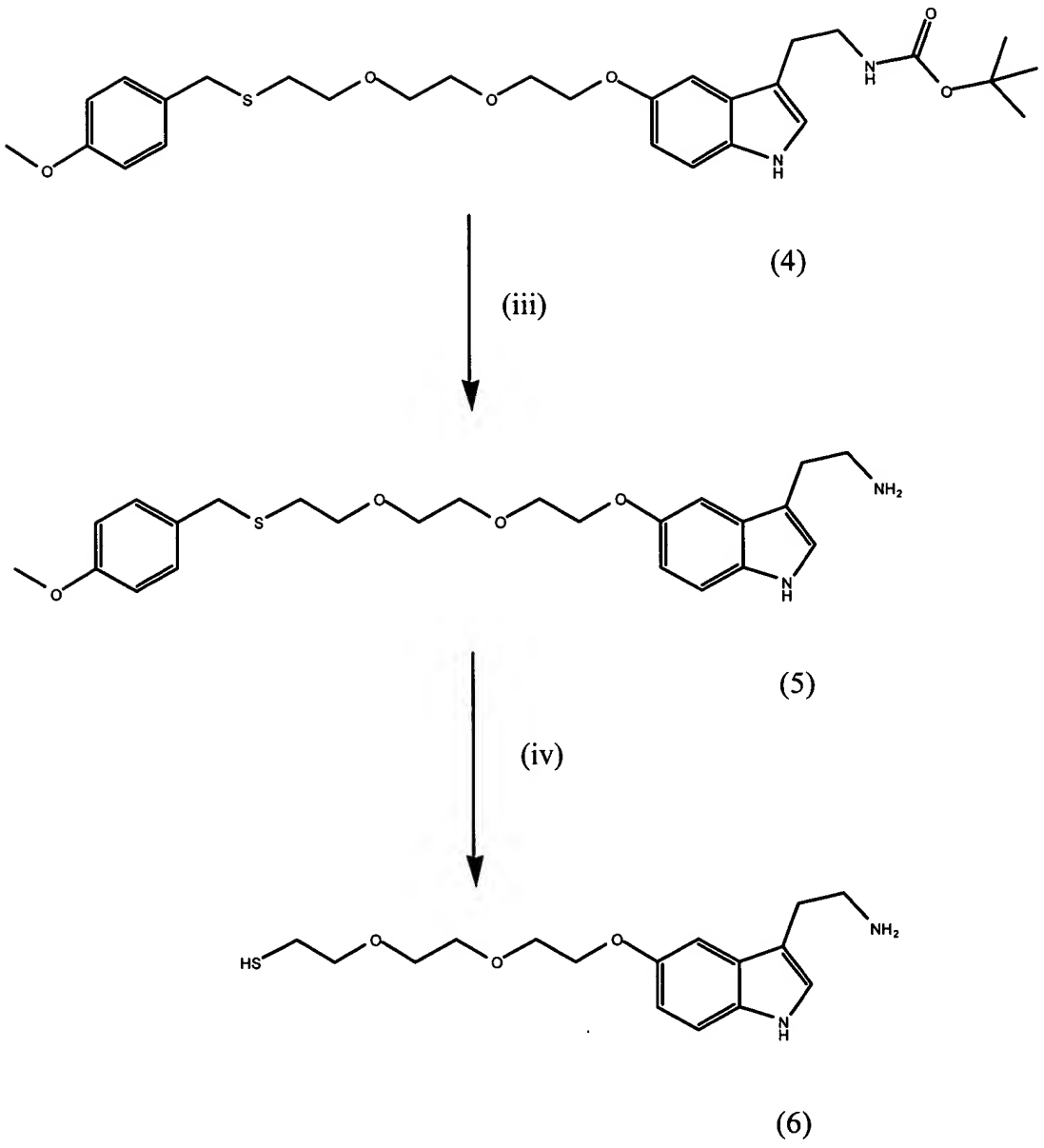

Chart 2. Method A

(i) Seratonin creatin sufate monohydrate, di-tertiarybutyl dicarbonate, potassium carbonate; (ii) 2, 3, Potassium carbonate (60 equiv), acetone, reflux 168 hours; (iii) 5, trifluoro acetic acid, room temperature, 2 hours; (iv) 5, Mercury(II) acetate, trifluoro acetic acid, hydrogen sulfide.

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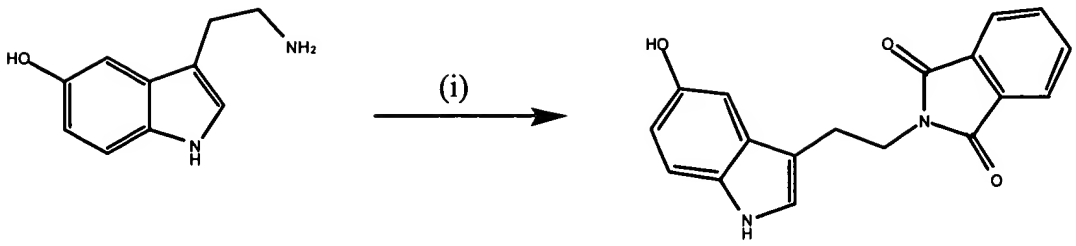

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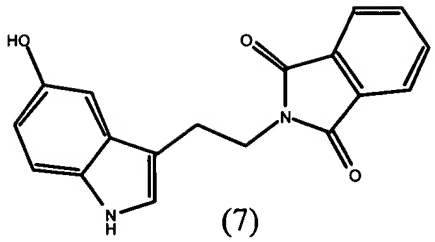

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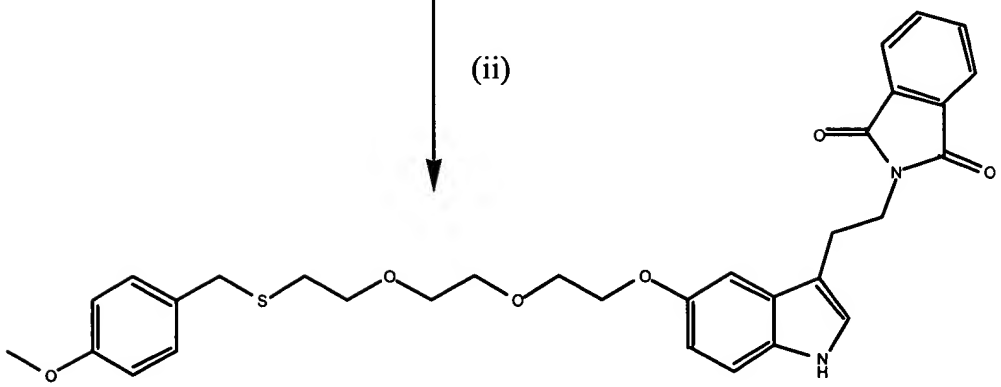
(ii)



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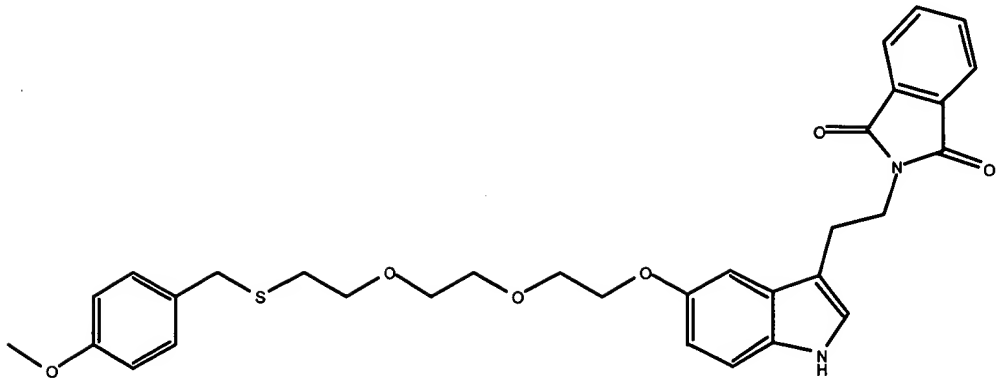

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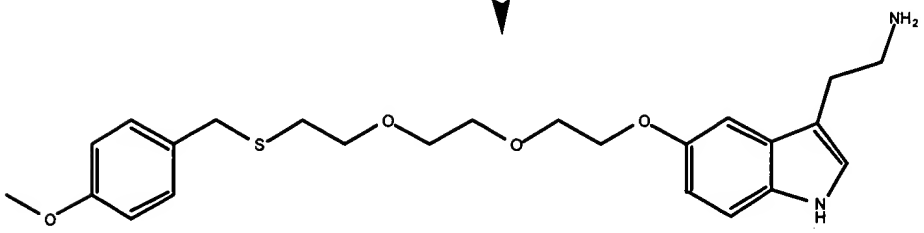
(ii)



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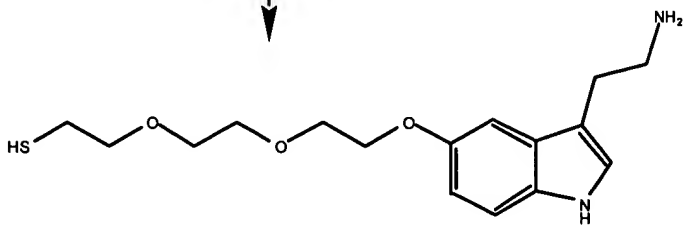


(6)

Chart 3. Method B

(i) Seratonin creatine sulphate monohydrate, N-Carbethoxyphthalimide; (ii) 7, 2, $CsCO_3$ (3 equivalents) reflux 24 hours; (iii) Hydrazine hydrate

(iv) Mercury(II) acetate, H_2S
The following compounds correspond with the compounds in charts 1, 2 and 3 above.

8-(4-methoxybenzylthio)-3,6-dioxaoctanol (1)

Sodium metal (0.8g, 34.8 mmols) is added to ethanol (100ml) in a 250ml round bottomed flask equipped with a reflux condenser and a stirrer. 4-Methoxy-α- toluenethiol (4.88ml, 34.8 mmol) is added upon complete reaction of the sodium with the ethanol. The mixture is stirred at room temperature for 30 minutes then 2-(2-(2-chloroethoxy)ethoxy)ethanol (5.88g, 34.8 mmols) is added. The reaction mixture is heated at reflux for 24 hours. After cooling to room temperature it is poured into saturated ammonium chloride solution (40ml) and extracted into dichloromethane (3x100ml). The dichloromethane solution is dried over magnesium sulfate and yields a yellow oil upon evaporation. The product is purified using column chromatography on silica eluted with a gradient system from dichloromethane to dichloromethane: methanol 10% to give approximately 7.24g (71%) of the product as a yellow oil.

8-(4-Methoxybenzylthio)-3,6-dioxaoctyl tosylate (2)

8-(4-methoxybenzylthio)-3,6-dioxaoctanol (7.24g, 25 mmols) is added to dry pyridine (5ml) and cooled to 0°C under nitrogen in a 100ml flask equipped with a stirrer. Para-toluene sulphonyl chloride (6.5g, 34 mmol) is slowly added to this solution and the mixture is stirred and allowed to warm to room temperature. It is stirred at room temperature for 18 hours after which it is added to water (100ml) and dichloromethane (100ml). The organic layer is separated and washed with hydrochloric acid (2N, 1x50 ml) and saturated sodium bicarbonate solution (50ml). It is dried over magnesium sulfate, filtered and evaporated. The crude product is obtained as a red oil and this is purified using column chromatography, in which the crude material is adsorbed onto silica and the column is eluted with a gradient system running from 20% diethyl ether: petroleum ether to 70% diethyl ether: petroleum ether. This yields approximately 8.00g (72%) of the product as a yellow oil.

3-[2-N-(tert-Butoxycarbonyl)amino]1H-indole-5-ol (3)

This compound is prepared as previous described in the Journal of medicinal chemistry 1996, 39, 314, Glennon R., et al. Potassium carbonate (1.3g, 9.5 mmols) is added all at once to a suspension of seratonin creatine sulfate monohydrate (1.9g, 4.7 mmols) dissolved in water (24ml), in a 100ml flask equipped with a stirrer. When the materials have dissolved di-tertbutyl dicarbonate (1.01g, 4.7 mmols) is added. The mixture is left stirring at room temperature for 24 hours. The product is extracted with ethyl acetate (3x20ml). The combined organic extracts are washed with water (1x20ml), hydrochloric acid (5%, 15ml) and brine (15ml). The organic solution is dried over magnesium sulfate and the crude product is obtained as a black tar upon evaporation. The product is purified using column chromatography on silica gel eluted with dichloromethane. This yields approximately 1.34g (100%) of the product as a pale yellow oil.

1-[3-[2-[N-(tert-Butoxycarbonyl)amino]ethyl]-1H-indol
-5-yloxyl-3,6-dioxa-8-(4-methoxybenzylthio)octane (4)

8-(4-Methoxybenzylthio)-3,6-dioxaoctyl tosylate (2.82g, 6.4 mmols) is added to acetone (100ml). 3-[2-N-(tert-Butoxycarbonyl)amino]1H-indole-5-ol (1.76g, 6.4 mmols) is dissolved in acetone (20ml) and the two solutions are

combined in a 250ml flask equipped with a reflux condenser and a stirrer. Dry potassium carbonated (60g) is added and the mixture is left refluxing for 168 hours. Upon cooling the solution is filtered and evaporated. The product is purified using column chromatography on silica gel eluted with a gradient system running from dichloromethane to 90% dichloromethane: methanol. This gave 1.9g (60%) of the product as a yellow oil.

1-[3-[2-aminoethyl]-1H-indol-5-yloxyl]-3,6-dioxa-8-(4-methoxybenzylthio) octane (5)

Method A:

1-[3-[2-[N-(tert-Butoxycarbonyl)amino]ethyl]-1H-indol-5-yloxyl-3,6-dioxa-8-(4-methoxybenzylthio)octane (0.8g, 1.5 mmols) is dissolved in toluene (60ml) in a 250ml round bottomed flask equipped with a stirrer. Trifluoro acetic acid (20ml) is added and the mixture is stirred at room temperature for 2 hours. It is evaporated under reduced pressure and the product is purified using silica gel chromatography eluted with triethylamine 3%: methanol 5%: dichloromethane 92%. This yields approximately 0.6g (92%) of the product (5) as a pale yellow oil.

Method B:

1-[3-[2-[N,N-Phtalimido]ethyl]-1H-indol-5-yloxyl]-3,6-dioxa-8-(4-methoxybenzylthio) octane (1.4g, 2.4 mmols) is dissolved in absolute ethanol (50ml) in a 100ml round bottomed flask equipped with a stirrer. Hydrazine mono hydrate (2ml) is added and the solution is stirred for 18 hours at room temperature and then evaporated. Dichloromethane (50ml) is added to the resulting tar and the mixture is heated at reflux for 30 minutes. After cooling it is filtered and evaporated and 7 was purified using silica gel column chromatography eluted with dichloromethane 95%: triethylamine 3%: methanol 2%. This yields approximately 0.6g (51%) of the product (5) as a pale yellow oil.

1-[3-[2-amino ethyl]-1H-indol-5-yloxy]-3,6-dioxa-8-mercapto octane (6)

1-[3-[2-aminoethyl]-1H-indol-5-yloxyl]-3,6-dioxa-8-(4-methoxybenzylthio) octane (0.6g, 1.4 mmols) is dissolved in trifluoroacetic acid (15ml) and cooled to 0°C in a 50ml round bottomed flask equipped with a stirrer. Anisole (1.5ml) and mercury (II) acetate (0.516g, 1.6 mmols) are added and the mixture is stirred at 0°C for 2 hours. The solution is evaporated and the resulting solid is washed with diethyl ether (3x50 ml). After air drying the solid is dissolved in glacial acetic acid (25ml) and hydrogen sulfide is bubbled through the solution for 30 minutes. Mercuric sulfide is removed by filtration and the solution is evaporated to dryness. The resulting oil is dissolved in dichloromethane and washed with sodium bicarbonate solution (1M, 1x20 ml). The solution is dried over

magnesium sulfate and evaporated. This yields approximately 0.072g (39%) as a pale yellow oil.

N,N-Phthalimido-2-(5-hydroxy-1H-indole-3-yl)ethylamine (7)

This compound is prepared using the method that has previously been described in the Journal of medicinal chemistry 1996, 39, 4717 Barf T., et. al. To a stirred solution of seratonin creatine sulphate monohydrate (3.8g, 9.4 mmols) in water (15ml) and tetrahydrofuran (15ml), is added a solution of 10% sodium bicarbonate until a pH of 8 is obtained.
N-Carbethoxyphthalimide is added and the mixture is stirred at room temperature overnight. The resulting solid is removed by filtration and it is re-crystallized from absolute ethanol, to give 2.5g (87%) of product as a yellow solid. Mp 215-216 ^{0}C (lit 213-216 ^{0}C).

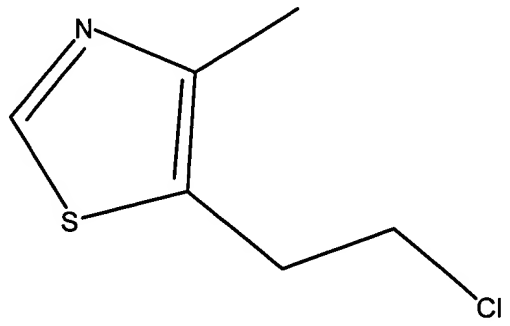
1-[3-[2-[N,N-Phtalimido]ethyl]-1H-indol-5-yloxyl]-3,6-dioxa-8-(4-methoxybenzylthio)octane (8)

8-(4-Methoxybenzylthio)-3,6-dioxaoctyl tosylate (1.6g, 3.6 mmols) is added to acetone (100ml) then N,N-Phthalimido-2-(5-hydroxy-1H-indole-3-yl)ethylamine (1g, 3.3 mmols) is added. Dry Cesium carbonate (3g, 3 equivalents) was added and the mixture is heated at reflux for 24 hours. The

solution is cooled to room temperature and filtered. The product (8) is purified using silica gel eluted with dichloromethane 98%: methanol. This gives approximately 1.1g of (8) as a pale yellow oil.

Attaching the linker arm to alkyl alcohols

The linker arms of the present invention may be attached to alkyl alcohols via an ether linkage. Many drugs, DNA, RNA, glycoproteins, intracellular messengers and hormones such as the steroids contain these functionalities. By way of an example a derivative of the neuroprotective agent chlormethiazole (9) has been synthesized.



(9)

A synthesis of the derivative of chlormethiazole is outlined in chart 4, below.

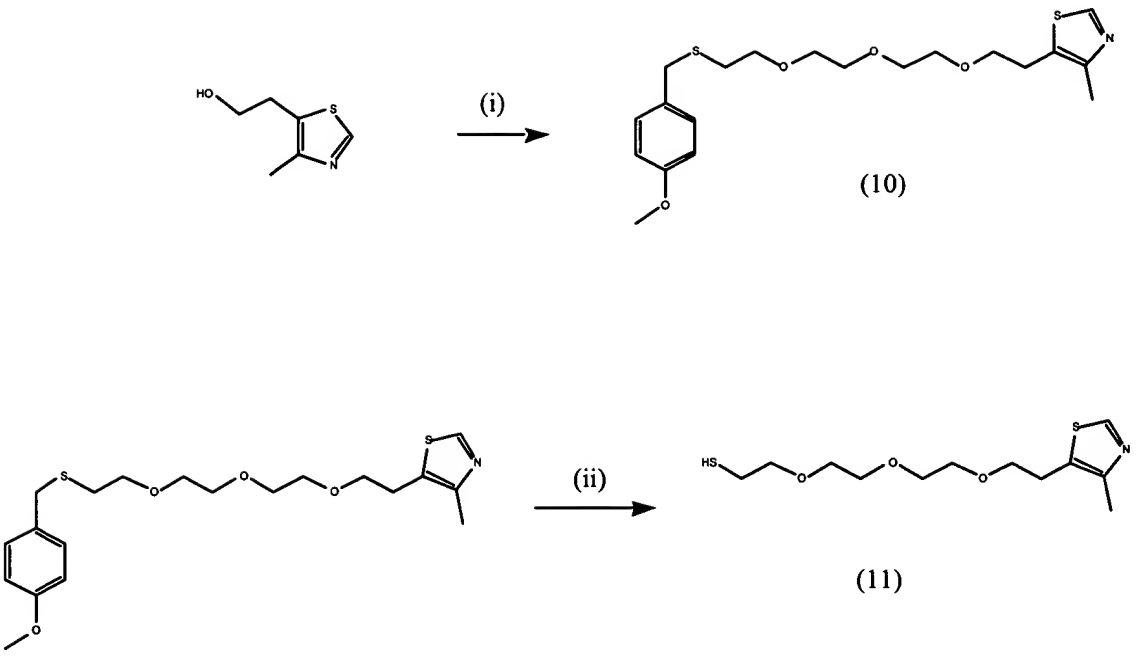


Chart 4. (i) 4-methyl-5-thiazoleethanol, 2, KOH, tertiary butyl ammonium chloride;

(ii) Mercury(II) acetate, trifluoroacetic acid, Hydrogen sulfide.

2-[2-[2-[2-(4-Methyl-thiazol-5-yl)-ethoxy]ethoxy]ethoxy]thioethyl-(4-methoxybenzyl)ether (10)

4-methyl-5-thiazoleethanol (1.5ml, 13.2 mmols) is added to dichloromethane (50ml). Potassium hydroxide (4g) dissolved in water (4ml) and tertiary butyl ammonium chloride (0.02g) are added. 8-(4-Methoxybezylthio)-3,6-dioxaoctyl tosylate (1.8g, 4.4 mmols) is dissolved on dichloromethane and added to the mixture. The mixture is heated at reflux for 240 hours and then cooled to room temperature. Water (20ml) is added, the organic layer is separated and dried over magnesium sulfate.

After removing the magnesium sulfate by filtration the solvent is removed under reduced pressure. The product is purified using column chromatography on silica gel eluted with ethyl acetate 99%: methanol. This yields approximately 0.33g (20%) of the product as a pale yellow oil.

2-[2-[2-[2-(4-Methyl-thiazol-5-yl)ethoxy]ethoxy]ethoxy]ethanethiol (11)

2-[2-[2-[2-(4-Methyl-thiazol-5-yl)-ethoxy]ethoxy]ethoxy]thioethyl-

(4-methoxybenzyl)ether (0.33g, 0.9 mmols) is dissolved in trifluoroacetic acid (10ml) and cooled to 0°C. When the solution is at a temperature of 0°C mercury (II) acetate (0.3g, 0.9 mmols) and anisole (1ml) are added and the mixture is stirred at 0°C for 2 hours. The solvent is evaporated under reduced pressure and the mercury salt is triturated with diethyl ether (3x50ml). The resulting solid is dissolved in glacial acetic acid (20ml) and hydrogen sulfide is bubbled through the solution for 30 minutes. After which the solution is filtered and evaporated under reduced pressure. The product is purified via column chromatography on silica gel eluted with dichloromethane 99%: methanol. This yields approximately 0.02g (8%) of the product as a colorless oil.

Alteration of linker arm to attach aryl and alkyl amines via an amide linkage

The polyethylene glycol linker arm can be altered so that it can be attached to aryl and alkyl amines via an amide linkage. The derivative of the linker arm can be readily prepared and a synthetic scheme for the derivative is outlined in chart 5, below.

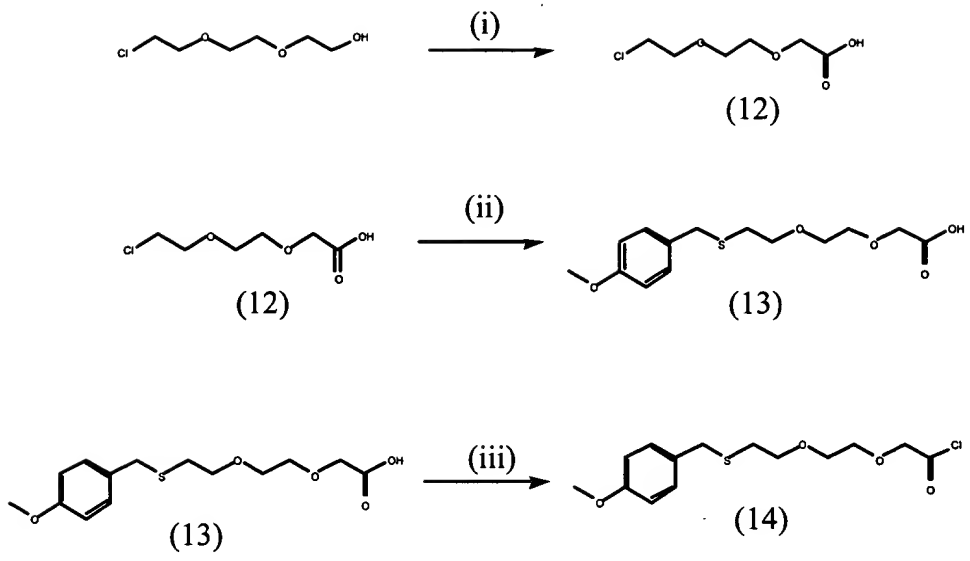
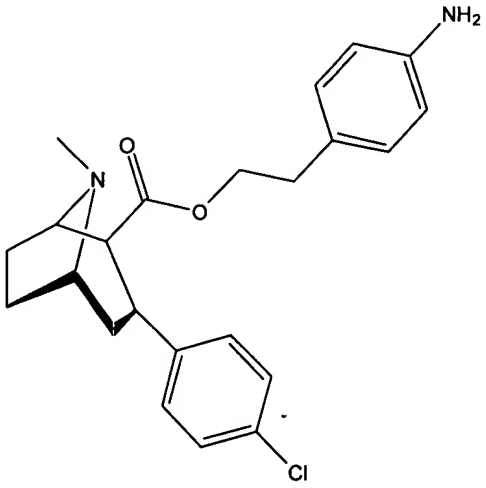


Chart 5.

(i) CrO_3, H_2SO_4; (ii) Na, ethanol, paramethoxy-α- toluene thiol, reflux 24 hours;
(iii) Oxalyl chloride, DMF

The resulting carboxylic acid (13) can be attached to amines using a variety of reagents such as DCC or by making the acid chloride (14).

Two such derivatives that we have synthesized are the derivative of the cocaine analogue RTI-4229-75 (15) and the derivative of GBR 12935 (16):



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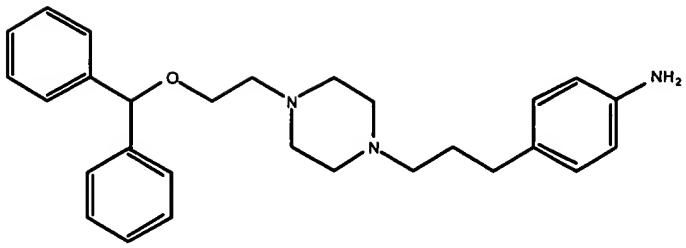

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The synthesis of these compound are outlined in charts 6, 7 and 8, below. The linker arm derivative that contains this carboxylic acid functionality may also be attached to proteins and antibodies via an amide bond, alternatively it may be attached to RNA and DNA via a ester linkage to the ribose or deoxy ribose moiety.

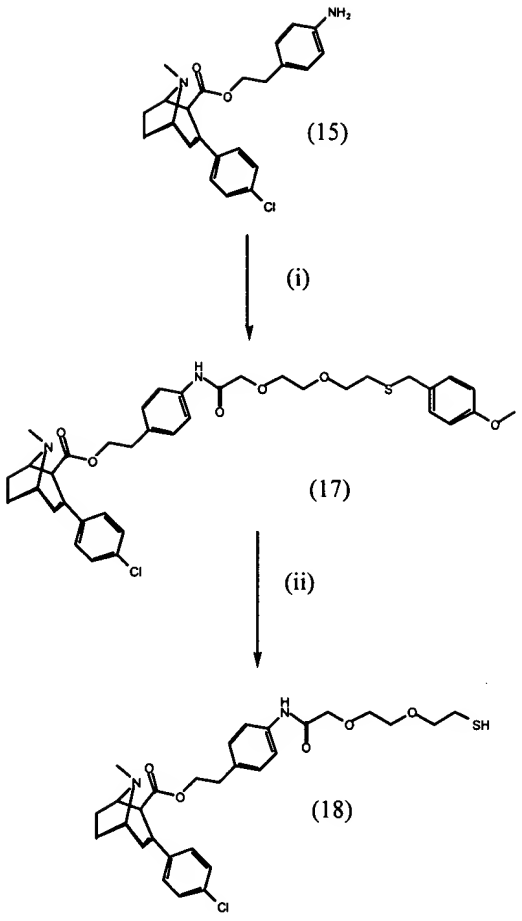
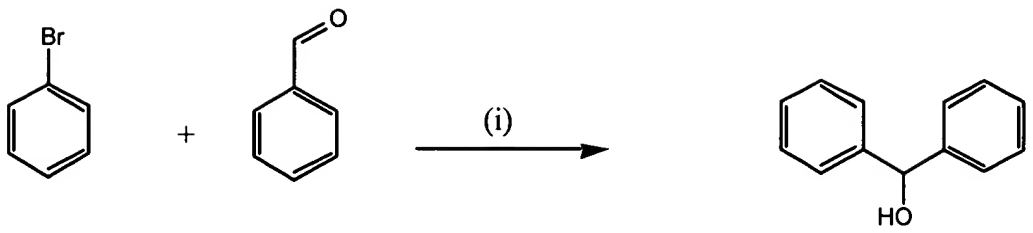


Chart 6.

(i) 16, triethylamine, dichloromethane; (ii) trifluoroacetic acid, mercury (II) acetate, glacial acetic acid, hydrogen sulfide



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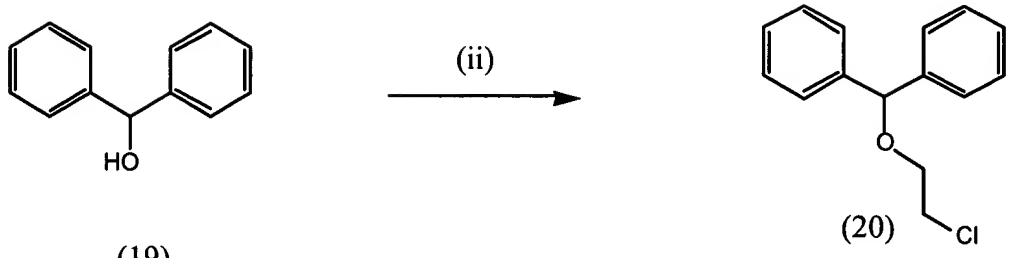

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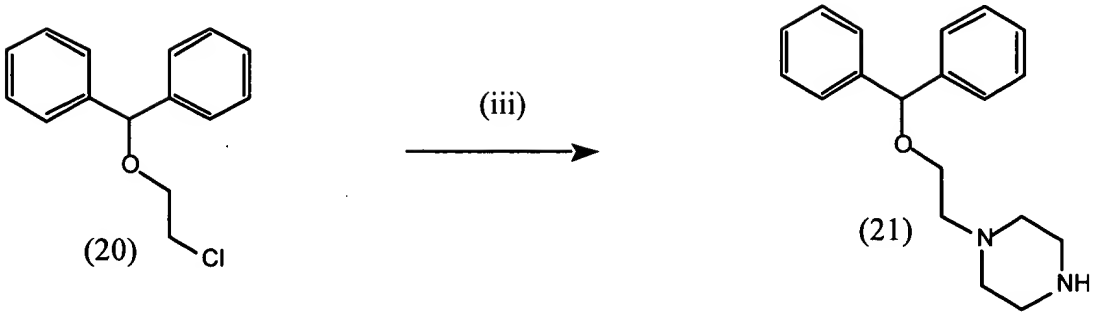

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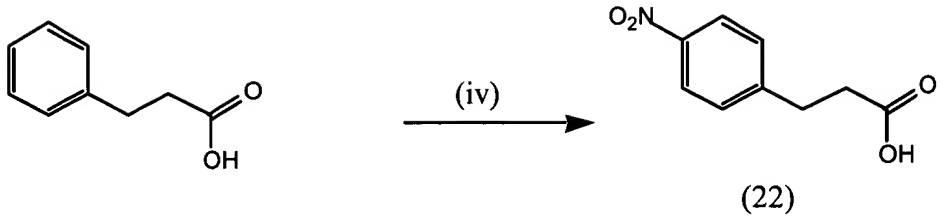

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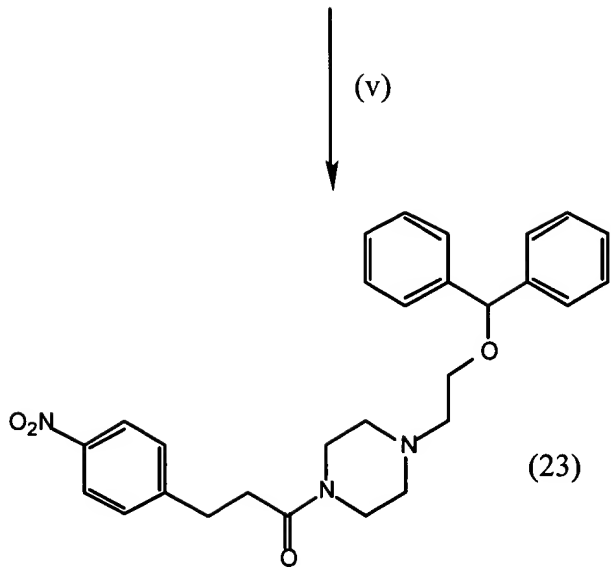

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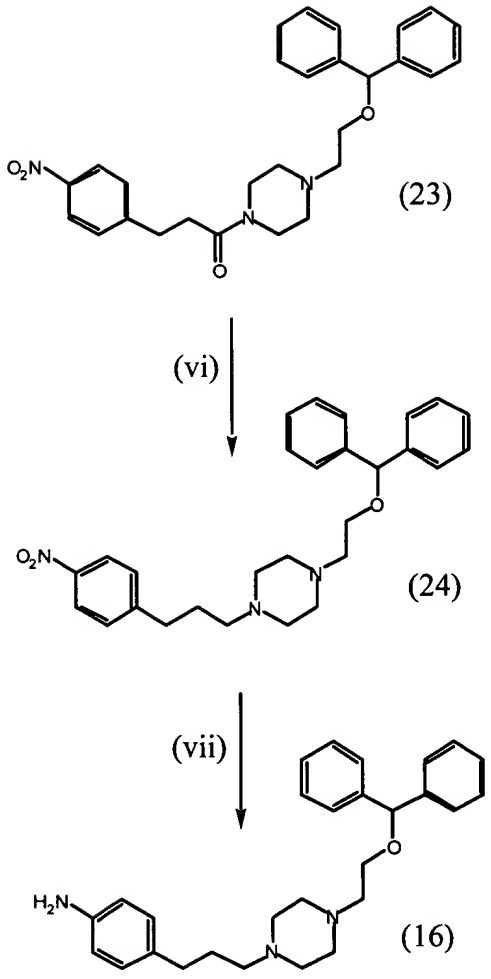

Chart 7.

(i) Mg, diethyl ether; (ii) 2-Chloroethanol, sulfuric acid;
(iii) piperazine hexahydrate, potassium carbonate; (iv) H_2SO_4, HNO_3;
(v) Oxalyl chloride, DMF, triethylamine; (vi) AlH_3; (vii) $SnCl_2$, CH_3CH_2OH

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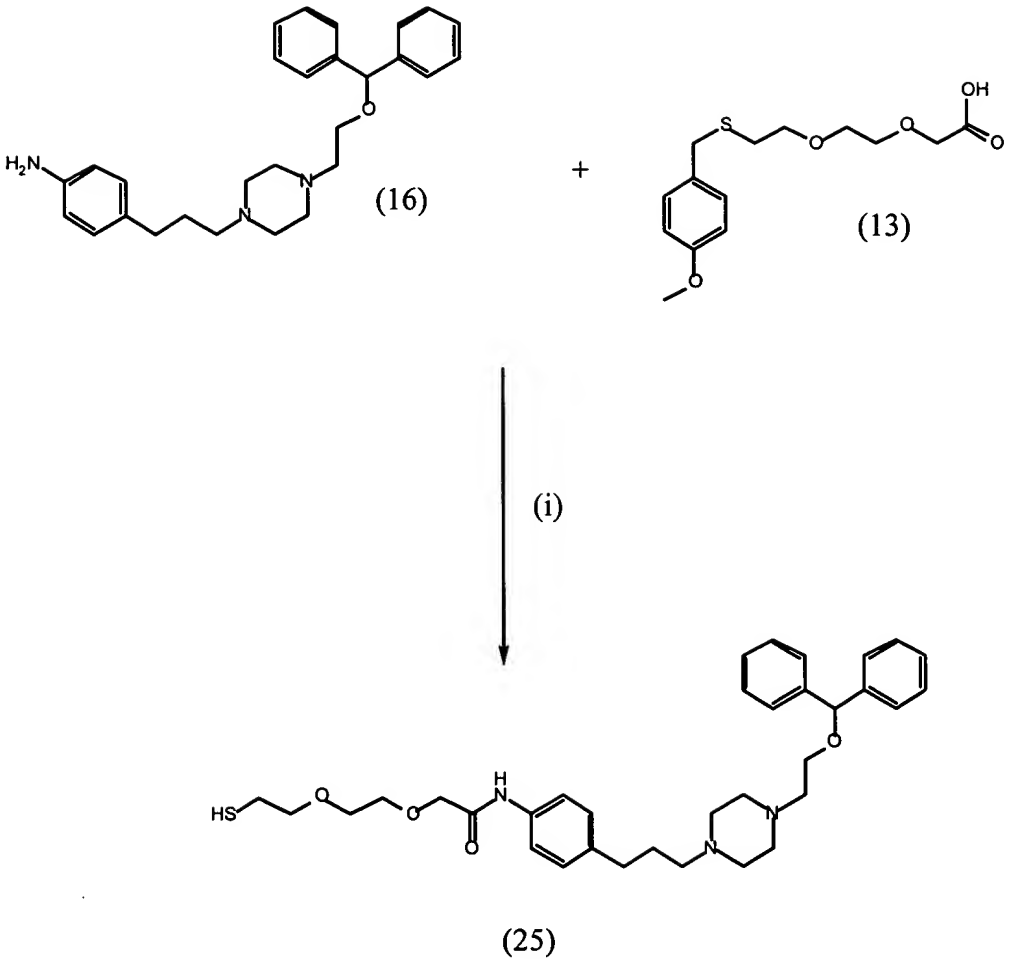


(25)

Chart 8.

(i) oxalyl chloride, DMF, dichloromethane, tetrahdrofuran, reflux 48 hours, silica, oxalic acid

2-(2-(2-Chloroethoxy)ethoxy)ethanoic acid (12)

2-(2-(2-chloroethoxy)ethoxy)ethanol (1.69g, 10 mmols) is dissolved in acetone (50ml). This solution is added drop wise to a solution of sulfuric acid (1.5M, 60ml) containing chromium (VI) oxide (5.79g, 38 mmols) at 0°C. Upon complete addition of the alcohol the solution is allowed to warm to room temperature for 18 hours. Inorganic chromium salts are removed by filtration and the solution is concentrated under reduced pressure. The crude product is extracted from solution using dichloromethane (3x100ml) and the combined extracts were dried over magnesium sulfate. After filtration and evaporation under reduced pressure the crude product is obtained as a colorless oil 1.7g (93%). This is used without further purification.

8-(4-Methoxybenzylthio)-3,6-dioxaoctanoic acid (13)

Sodium (0.253g, 11 mmols) is added to absolute ethanol (50ml) and stirred at 0°C for 30 minutes. 4-Methoxy-α-toluenethiol (0.78ml, 6 mmols) is added and the mixture is stirred at room temperature for 30

minutes. 2-(2-(2-Chloroethoxy)ethoxy)ethanoic acid (1g, 5.5 mmols) is added and the mixture is heated at reflux for 18 hours. It is cooled to room temperature poured into distilled water (100ml) and acidified with hydrochloric acid (2M, 1X50 ml). The product is extracted with dichloromethane (2X100ml) and the organic solution is dried over magnesium sulfate. After filtering the organic solution it is evaporated under reduced pressure. The product is purified using column chromatography on silica eluted with a gradient system running from dichloromethane to dichloromethane 90%: methanol. This gives approximately 1.24g (94%) of the product as a colorless oil.

1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)piperazine (16)

1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-nitrophenyl)-1-oxopropyl)piperazine (0.9g, 2.8 mmols) is dissolved in absolute ethanol (10ml) in a 100ml round bottomed flask equipped with a stirrer and reflux condenser. Tin (II) chloride dihydrate (2.6g) is added and the mixture is heated at reflux for 90 minutes. The solution is poured into crushed ice and a solution of sodium carbonate (5%) in water is added until a pH of 8 is obtained. The aqueous solution is extracted with ethyl acetate (3x200ml) and this is dried over magnesium sulfate. The product is purified using column chromatography on silica gel eluted with

ethylacetate 92%: methanol 5%: triethylamine. This gives approximately 0.66g (78.6%) of the product as a pale yellow oil.

3-(4-Chlorophenyl)-8-methyl-8aza-bicyclo[3.2.1]octane-2-carboxylic acid 2-[4-(2-{2-[2-(4-methoxybenzylthio)ethoxy]ethoxy}acetylylamino)phenyl]ethyl ester (17)

8-(4-Methoxybenzylthio)-3,6-dioxaoctanoic acid (0.008g, 0.027 mmols) is dissolved in dry toluene (10ml), oxalyl chloride (0.0008ml is added and then dry dimethyl formamide (1 drop).The mixture is stirred at room temperature for 1 hour and then evaporated to yield crude 8-(4-Methoxybenzylthio)-3,6-dioxaoctonyl chloride (14). The 8-(4-Methoxybenzylthio)-3,6-dioxaoctonyl chloride (14) is dissolved in dry dichloromethane (20ml), 3β-(p-Chlorophenyl)tropane-2β-carboxylic acid p-aminophenylethyl ester (0.0100g, 0.025 mmols) and triethylamine (2 drops) are added. The mixture is heated at reflux for 18 hours cooled and evaporated under reduced pressure. (17) is purified using column

chromatography on silica gel eluted with ethyl acetate 98%: triethyl amine. This yields approximately 0.006g (34%) of (17) as a tar.

3-(4-Chlorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid 2-(4-{2-[2-(2-mercaptoethoxy)ethoxy]acetylamino}phenyl)ethyl ester (18)

3-(4-Chlorophenyl)-8-methyl-8aza-bicyclo[3.2.1]octane-2-carboxylic acid 2-[4-(2-{2-[2-(4-methoxybenzylthio)ethoxy]ethoxy}acetylylamino)phenyl]ethyl ester (0.021g, 0.03 mmols) is dissolved in trifluoroacetic acid (5ml) and cooled to 0°C. Anisole (0.05ml) and mercury (II) acetate (0.011g, 0.036 mmols) are added to this solution and it is stirred at 0°C for 2 hours. The solvent is evaporated, the product is triturated with diethyl ether and vacuum dried. Then it is dissolved in glacial acetic acid (10ml) and hydrogen sulfide is bubbled through the solution for 30 minutes. The solution is filtered evaporated and methanolic hydrogen chloride (10ml) is added to the tar. Then it is evaporated under reduced pressure and this procedure

is repeated 5 more times. After drying under vacuum 0.008g (44%) of (17) is obtained as the hydrochloride salt.

Diphenylmethanol (19)

This compound was synthesised using the method reported in the Journal of the Chemical Society, 1960, 2133, by Mole. A 1 litre three necked round bottomed flask equipped with a stirrer a reflux condenser and a 200ml pressure equalising addition funnel, is charged with magnesium turnings (15.36g, 630 mmols). Dry diethyl ether (150ml) and iodine (0.1g, 0.3 mmols) are added. The mixture is heated at reflux until the purple iodine colour disappeared and to this solution was added 5ml of a solution of bromo benzene (65.2ml, 97.24g, 620 mmols) in 150ml of anhydrous ether. The reaction mixture is heated at reflux until a cloudy grey color forms. The heat is removed and the remaining bromo benzene is added drop wise at such a rate so as to maintain reflux. The solution is heated at reflux for a further hour after the addition of bromobenzene is complete. After which it is cooled to 10^{0}C in an ice acetone bath and benzaldehyde (60ml, 62.4g, 588 mmols) in anhydrous ether 200ml is added drop wise so that the temperature of the reaction mixture does not exceed 20^{0}C. The reaction mixture is allowed to warm to room temperature after the addition of benzaldehyde and it is stirred at room temperature for a further 18 hours. The reaction is quenched by adding ammonium chloride solution

(100ml) at 0°C. After which the organic layer is separated washed with water and dried over magnesium sulfate. The solution is filtered and evaporated and the product is washed with hexanes (150ml) to give approximately 51.6g (45%) of the product as a colorless solid mpt = 64 – 64.5°C.

1,1'-[(2-Chloroethoxy)methylene]bis-benzene (20)

This compound was synthesized using the method reported in the European Journal of Medicinal Chemistry, 1980, 15 (4), 363, by Van Der Zee P. et. al. Freshly distilled 2-chloroethanol (11g) is added to toluene (25ml) in a 500ml round bottomed flask, equipped with a reflux condenser a 200ml pressure equalizing funnel and a stirrer. Concentrated sulphuric acid (1ml) is added and the mixture is heated at reflux for 5 hours during which Diphenylmethanol (11,56g, 90 mmols) dissolved in toluene (150ml) is added drop wise. Then the solution is cooled to room temperature the aqueous layer is separated and the organic solution is washed with sodium bicarbonate (sat, 100ml) and water (2 x 100ml). It is dried over magnesium sulphate filtered and evaporated. The product is purified by vacuum distillation and this gives approximately 6g (26%) of the product as a colorless oil.

1-[2-[bisphenylmethoxy]ethyl]piperazine (21)

Piperazine hexahydrate (47g, 240 mmols) is added to toluene (100ml) and anhydrous potassium carbonate (66g, 600 mmoles) is added. The mixture is heated at reflux and 1,1'-[(2-Chloroethoxy)methylene]bis-benzene (20g, 80 mmols) is added drop wise over five hours. After refluxing for a further 18 hours the solution is allowed to cool to 70°C washed with water (5 x 250ml), dried over magnesium sulfate, filtered and evaporated. The resulting yellow oil is converted to a dimaliate salt by crystallising from diethyl ether. This gives approximately 25g (50%) of the product as a colourless solid.

Para-Nitrohydrocinnamic acid (22)

This compound is prepared using the method described by Moloney in the journal of medicinal chemistry, 1999, volume 42 No 14 page 2504. Hyrocinnamic acid is added to concentrated sulfuric acid (49ml) in a 3 necked 250ml round bottomed flask equipped with a stirrer and thermometer. The flask is cooled to 0°C in an ice bath and concentrated nitric acid (10ml) is added drop wise maintaining the temperature below 10°C. The solution is stirred for a further hour at 0°C after the complete addition of the nitric acid. Then the ice bath is removed and the mixture is stirred at room temperature for 30 minutes.

The resulting orange solution is poured into ice and the crude product is collected by filtration. The product is air dried and re-crystallised from ethyl acetate giving approximately 10g (29%) of the para-nitrohyrocinnamic acid as a colorless solid.

1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-nitrophenyl)-1-oxopropyl)piperazine (23)

para-Nitrohyrocinnamic acid (2.8g, 9.5 mmols) is added to dry toluene (100ml), in a 250ml round bottomed flask equipped with a stirrer and a reflux condenser. Oxalyl chloride (1ml) is added, after which a catalytic quantity of dry DMF (2drops) is also added and the mixture is stirred at room temperature for 2 hours. The solvent is removed by evaporation and the crude acid chloride is dissolved in dry dichloromethane (100ml). Dry triethylamine (10ml) and 1-[2-[bisphenylmethoxy]ethyl]piperazine (1.84g, 9.5 mmols) are dissolved in dry dichloromethane (50ml) and added to the solution of p-nitrohyrdrocinnamyl chloride. The mixture is heated at reflux for 18 hours under argon in a 250ml round bottomed flask equipped with a stirrer and reflux condenser. The solvent is removed under reduced pressure and the product is purified using silica gel chromatography eluted with dichloromethane 96%: methanolic ammonia. The resulting yellow oil which is converted into the yellow maleate salt by crystallisation from diethyl ether. This gives approximately 4.3g (99%) of the product.

1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-nitrophenyl)propyl)piperazine (24)

1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-nitrophenyl)-1-oxopropyl)piperazine (5g, 14.8 mmols) in a 250ml round bottomed flask equipped with a stirrer and a reflux condenser is dissolved in dry THF (100ml). Alane in toluene (0.5M, 59ml) is added and stirred at room temperature for 30 minutes. The reaction is quenched with sodium hydroxide solution (10%, 200ml). The aqueous solution is extracted with diethyl ether (3x150ml), dried over magnesium sulfate filtered and evaporated. The product is purified using silica gel chromatography eluted with a gradient system eluted with ethyl acetate 90%: methanol to ethyl acetate 87%: methanol 10%: triethylamine. This gives approximately 3.35g (68%) of the product as a pale yellow oil.

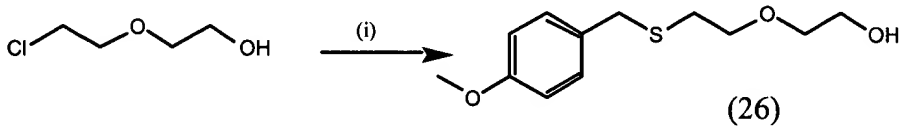
N-(4-(3-[4-(2-Benhydryloxyethyl)piperazine-1-yl]propyl)phenyl-2-[2-(2-mercaptoethoxy)ethoxy] acetamide (25)

8-(4-Methoxybenzylthio)-3,6-dioxaoctanoic acid (0.6g, 2.2 mmols) is dissolved in dry toluene (50ml) under nitrogen in a 100ml round bottomed flask equipped with a stirrer and a reflux condenser. Oxalyl chloride (0.5ml) and a catalytic quantity of dimethyl formamide (1 drop) are added. The solution is stirred at room temperature for 2 hours, then evaporated under reduced

pressure. The resulting crude 8-(4-Methoxybenzylthio)-3,6-dioxaoctonyl chloride (14) is dissolved in dry dichloromethane (100ml) in a 250ml round bottomed flask equipped with a reflux condenser and a stirrer. 1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)piperazine (0.66g, 2.2 mmols) in dry tetrahydrofuran and triethylamine (5ml) are added and the mixture is allowed to reflux under nitrogen. Then the solvent is evaporated and the product is columned on silica eluted with ethyl acetate 93%: methanol 5%: triethylamine. The crude product is converted to the oxylate salt by dissolving it in methanol (50ml) and adding oxalic acid (1g) dissolved in methanol (20ml). The resulting solid is left standing at room temperature for 18 hours and removed by filtration. The oxalate salt is converted back to the base and the product is purified by column chromatography on silica eluted with a gradient system running from dichloromethane (90%): methanol to dichloromethane (87%): methanol 10%: triethylamine. This gives the product as a yellow oil it is converted back to the oxylate salt, as described above and this is filtered and air dried. To yield approximately 0.28g (28.9%) of the product as a yellow solid.

Varying the length of the linker arm

The length of the linker arms of the present invention may be changed. Accordingly, at least a di and tetra polyethylene glycol linker arm may be synthesised. The synthetic routes for these compounds are outlined in charts 9 and 10, below. The linker arm is shortened in chart 9 and lengthened in chart 10.



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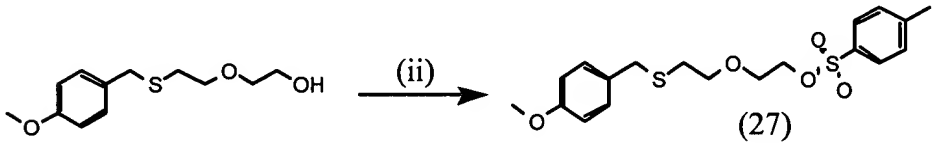

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Chart 9.

(i) Sodium, ethanol, paramethoxy-α-toluene thiol, reflux 24 hours;

(ii) pyridine, para toluene sulphonyl chloride, stir at room temperature for 24 hours.

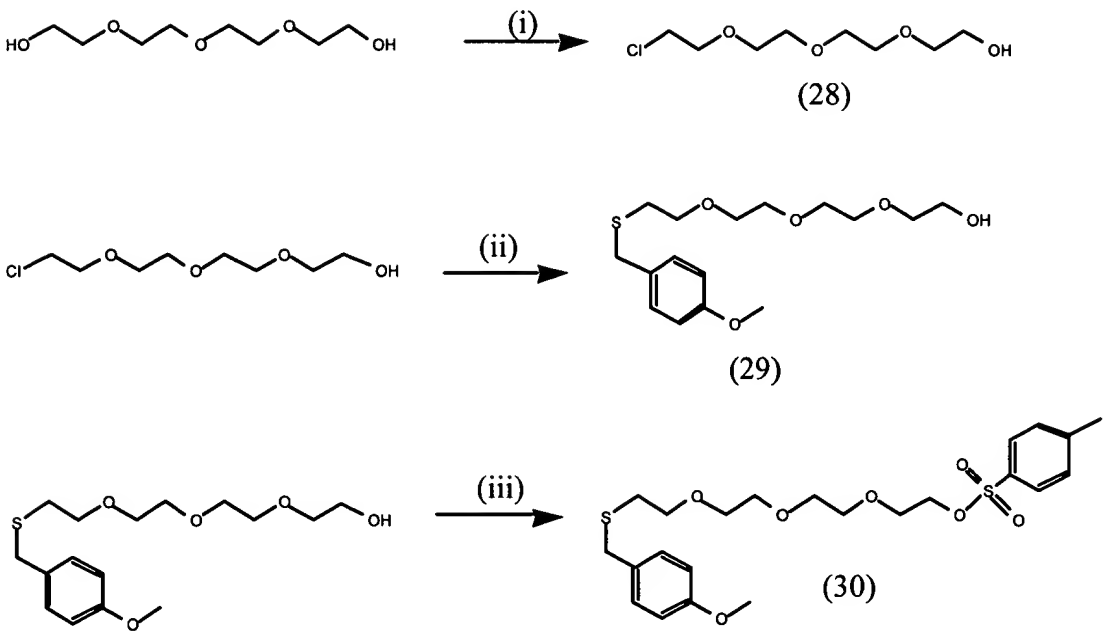


Chart 10.

(i) thionyl chloride; (ii) Sodium, ethanol, paramethoxy-α-toluene thiol, reflux 24 hours; (iii) pyridine, para-toluene sulfonyl chloride, stir at room temperature for 24 hours.

5-(4-methoxybenzthio)-3-oxapentanol (26)

Sodium metal (0.92g, 40 mmols) is added to absolute ethanol (100ml) at 0°C.

4-methoxy-α-toluenethiol (5.6ml) is added after the sodium has completed reacting.

2-(2-chloroethoxy)ethanol (5.48g, 44 mmols) is added 30 minutes later and the mixture is heated at reflux for 18 hours. The solution is cooled to room temperature and added to saturated ammonium chloride solution (100ml). It is extracted into dichloromethane (3x100ml). After drying the combined organic extracts over magnesium sulfate and filtering, the dichloromethane is removed under reduced pressure. The product is purified by column chromatography on silica gel eluted with a gradient system from dichloromethane to dichloromethane 90%: methanol. This yields approximately 5.9g (60%) of the product as a colorless oil.

5-(4-Methoxybenzylthio)-3-oxapentyl tosylate (27)

5-(4-Methoxybenzylthio)-3-oxapentanol (26), (2.42g, 10 mmols) is added to dry pyridine (10ml) and cooled to 0°C, para-toluene sulfonyl chloride (2.59g, 14 mmols) is added and the mixture is allowed to warm to room temperature over an 18 hour period with stirring. Water (50ml) and dichloromethane (100ml) are added. The organic layer is separated, it is washed with hydrochloric acid (2M, 1x100ml) and water (50ml). The organic solution is dried over magnesium sulfate, filtered and evaporated. The product is purified using column chromatography on a silica column eluted with a gradient system from petroleum spirit 70%: diethyl ether to petroleum spirit 30%: diethyl ether. This yields approximately 0.13g (3.6%) of the product as a colorless oil.

2-(2-(2-(2-Chloroethoxy)ethoxy)ethoxy)ethanol (28)

Tetraethylene glycol (192g, 990 mmols) is added to dry chloroform (200ml) in a 1L flask equipped with a stirrer, reflux condenser and a thermometer. Dry pyridine (80ml) is added to this solution and it is cooled to 0°C. Freshly distilled thionyl chloride (73ml) is added over a 4 hour period, whilst maintaining the temperature below 10°C. After all the

thionyl chloride has been added the solution is heated at reflux for 18 hours. Then the chloroform is removed under reduced pressure and the resulting residue is extracted with water (2x100ml). The aqueous solution is washed with hexane's (2x100ml) and the crude product is extracted into toluene (5x100ml). Then the solvent is dried with magnesium sulfate filtered and evaporated. The product is purified by distillation under reduced pressure using an aspirator (Bpt = 140-160°C). This yields approximately 19.8g (9.4%) of the product as a colorless oil.

11-(4-Methoxybenzylthio)-3,6,9-trioxaundecanol (29)

Sodium metal (0.8g) is added to absolute ethanol (100ml) at 0°C in a 250ml round bottomed flask equipped with a stirrer and a reflux condenser. After the sodium has completely reacted with the ethanol 4-methoxy-α-toluenethiol (4.9ml) is added. This is stirred at room temperature for 30 minutes.

Then 2-(2-(2-(2-Chloroethoxy)ethoxy)ethoxy)ethanol (7.4g, 34 mmols) is added and the mixture is heated at reflux for 18 hours. After cooling to room temperature it is added to saturated ammonium carbonate solution (100ml) and extracted into dichloromethane (3x100ml). The combined organic extracts were dried over magnesium sulfate filtered and

evaporated. The product is purified by column chromatography on silica gel eluted with a gradient system from dichloromethane to dichloromethane 90%: methanol. This yields approximately 6g (53%) of the product as an oil.

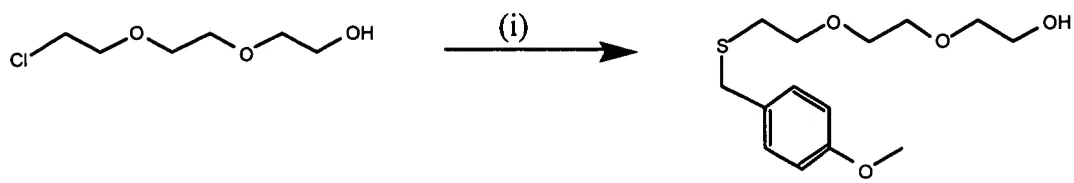
11-(4-Methoxybenzylthio)-3,6,9-trioxaundecanyl tosylate (30)

2-(2-(2-(2-Chloroethoxy)ethoxy)ethoxy)ethanol (5.9g, 18 mmols) is dissolved in dry pyridine (10ml) and cooled to 0°C, in a 50ml round bottomed flask equipped with a stirrer and a calcium chloride drying tube. Para-toluene sulfonyl chloride (4.66g, 24 mmols) is added to the mixture and it is stirred for 18 hours, during this period the temperature of the reaction mixture is allowed to increase from 0°C to room temperature. Water (50ml) is added to the reaction mixture and it is extracted with dichloromethane (2x50ml). The combined organic extracts are washed with hydrochloric acid (2M, 2x50ml), saturated sodium bicarbonate solution (2x50ml) and water (2x50ml). After which the solution is dried over magnesium sulfate, filtered and evaporated. The product is purified using column chromatography on a gradient system

from ethyl acetate 40%: hexane to ethyl acetate. This yields approximately 6.7g (77.8%) of the product as a colorless oil.

Linker arm synthesis with other functionalities

The linker arms of the present invention may be synthesized with other functionalities, including a chloride and an amine functionality. The synthesis of these compounds is outlined in chart 11, below. The amino functionality may also be attached to drugs or biologically active molecules such as cholesterol, proteins and antibodies, via an amide linker.



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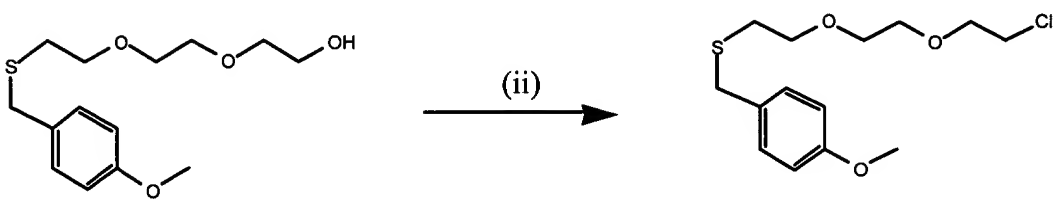

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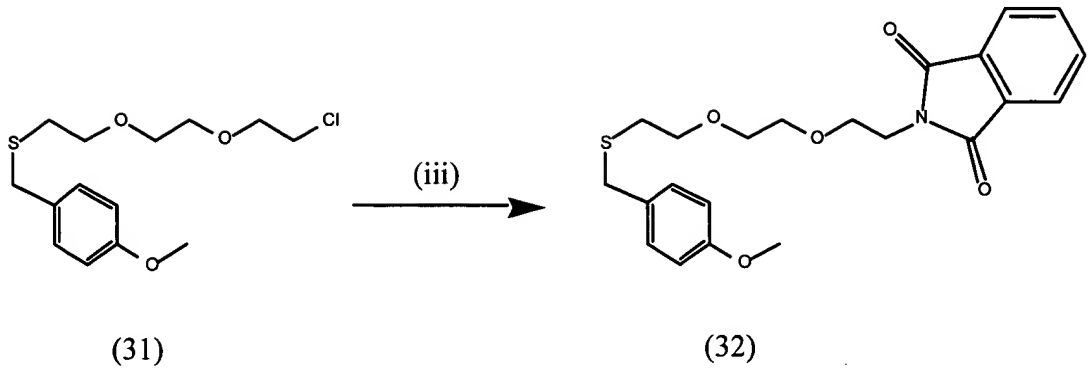

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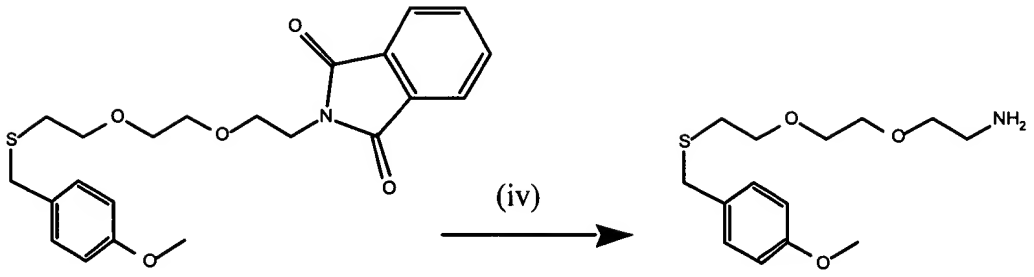


(32) (33)

Chart 11.

(i) sodium, ethanol, para-methoxy-α-toluene thiol, reflux 24 hours;
(ii) thionyl chloride, pyridine; (iii) potassium pthalimide; (iv) hydrazine

8-(4-Methoxybenzylthio)-3,6-dioxaoctyl chloride (31)

8-(4-Methoxybenzylthio)-3,6-dioxaoctanol (1.72g, 6 mmols) is dissolved in dry dichloromethane (30ml) and dry pyridine (0.97ml, 12 mmols) is added. The solution is stirred for 5 minutes then thionyl chloride (0.5ml) is added. The mixture is heated at reflux overnight. Then it is poured into hydrochloric acid (2M, 25ml) and the organic layer is separated. The aqueous solution is extracted with dichloromethane (2x25 ml) and the combined organic extracts are washed with water (10ml). After drying over magnesium sulfate the solution is filtered and evaporated. The product is purified using silica gel chromatography eluted with a gradient system from petroleum ether 60%: diethyl ether to petroleum ether 40%: diethyl ether. This yields approximately 0.39g (57%) of the product as a colorless oil.

8-(4-Methoxybenzylthio)-1-(N-phthalimido)-3,6-dioxaoctane (32)

8-(4-Methoxybenzylthio)-3,6-dioxaoctyl chloride (0.53g, 1.7 mmols) is dissolved in dimethyl formamide and potassium phthalimide (0.32g, 2 mmols) is added. The mixture is heated at 100°C for 18 hours and then cooled to room temperature. It is poured into water (100ml) and

extracted with diethyl ether (3x100 ml), after drying over magnesium sulfate it is filtered and evaporated. The product is purified using silica gel chromatography eluted with a gradient system from petroleum ether 60%: diethyl ether to petroleum ether 40%: diethyl ether. This gave 0.39g (57%) of the product as a colorless oil.

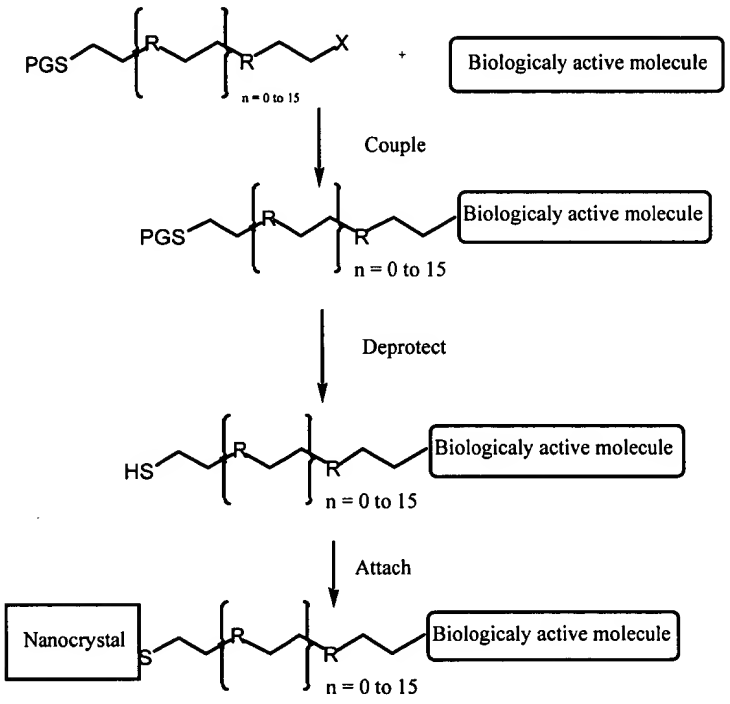
8-(4-Methoxybenzylthio)-3,6-dioxaoctylamine (33)

8-(4-Methoxybenzylthio)-1-(N-phthalimido)-3,6-dioxaoctane (0.39g, 0.96 mmols) is dissolved in absolute ethanol and hydrazine hydrate (1ml) is added. The mixture is heated at reflux for 1 hour and the solvent is removed under reduced pressure.

Water (10ml) and sodium hydroxide solution (1M, 10ml) are added to the resulting tar and the product is extracted with diethyl ether (3x50ml). The ethereal solution is dried over magnesium sulfate filtered and evaporated to yield approximately 0.25g (92%) of the product as an oil.

Attachment of biologically active compounds to the linker arm

A biologically active organic compound may be attached to the linker arm as follows:



Where X is Cl, Br, I, OTs, OMs, OTf, NH_2, SH, OH, C=O, COCl, CO_2H, etc.

The biologically active molecule is attached to the linker arm via a functional group or a methylene group. R may be O, NH, S, CH_2, etc. PG is a protecting group and may be para-methoxy benzyl, benzyl, a thioamide, a thio ether, etc.

Attaching linker arms to nanocrystal core shells

This example discloses a method of attaching linker arms of the present invention to nanocrystal core shells. An example of the methodology used is outlined below:

9 mg of trioctylphosphine oxide coated core shells are weighed out and suspended in pyridine (2ml). The concentration and thus the number of moles of nanocrystals may be determined before hand using UV-vis spectroscopy. This suspension is stirred at 60°C for 24 hours, N-(4-(3-[4-(2-Benhydryloxyethyl)piperazine-1-yl]propyl)phenyl-2-[2-(2-mercaptoetoxy)ethoxy] acetamide (25), (100mg) is dissolved in dichloromethane (100ml) and 2.7ml of this solution is added to the solution of nanocrystals. This gives approximately 100 ligands per core shell. The solution is stirred at 60°C under argon for 2 hours. Upon cooling to room temperature the solution is added to hexanes. Ligand coated core shells crystallise out of solution and are collected by filtration.

The water solubility of the ligand functionalised core shells may be increased if necessary by using a modification of the method of Fred Mikulec (private communication). Mercaptoacetic acid (1ml) and dimethyl formamide (1ml) are added to the ligand coated core shells and stirred at room temperature under argon for 2 hours. After cooling to room temperature the solution is diluted with dimethyl formamide (100ml) and potassium teriary butoxide (1.61g) is added. The resulting solid is collected by centrifugation and is washed with tetrahydrofuran (4 x 100ml) and methanol (7 x 100ml). The product is collected by centrifugation to yield 45 mg of 1-[2-bisphenylmethoxy]ethyl]-4-(3-(4-(3,6-dioxa-8-thiol)octanamidophenyl)propyl piperazine (25) coated nanocrystals. After drying the precipitate under reduced pressure for 4 days at room temperature the ligand coated cores can be dissolved in a minimum quantity of buffer in a pH range of 6 to 8.

This invention thus being described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as would be obvious to one of ordinary skill in the art are intended to be included within the scope of the following claims.

All cited patents and publications referred to in this application are herein expressly incorporated by reference.